

## METHODS AND COMPOSITIONS FOR USE OF INFLAMMATORY PROTEINS IN THE DIAGNOSIS AND TREATMENT OF METABOLIC DISORDERS

### Background of the Invention

[0001] Obesity represents the most prevalent of body weight disorders, affecting an estimated 30 to 50% of the middle-aged population in the western world. Obesity, defined as a body mass index (BMI) of 30 kg/m<sup>2</sup> or more, contributes to diseases such as coronary artery disease, hypertension, stroke, diabetes, hyperlipidemia and some cancers. (See, e.g., Nishina, P.M. *et al.* (1994), *Metab.* 43:554-558; Grundy, S. M. & Barnett, J.P. (1990), *Dis. Mon.* 36:641-731). Obesity is a complex multifactorial chronic disease that develops from an interaction of genotype and the environment and involves social, behavioral, cultural, physiological, metabolic and genetic factors.

[0002] Generally, obesity results when energy intake exceeds energy expenditure, resulting in the growth and/or formation of adipose tissue via hypertrophic and hyperplastic growth. Hypertrophic growth is an increase in size of adipocytes stimulated by lipid accumulation. Hyperplastic growth is defined as an increase in the number of adipocytes in adipose tissue. It is thought to occur primarily by mitosis of pre-existing adipocytes caused when adipocytes fill with lipid and reach a critical size. An increase in the number of adipocytes has far-reaching consequences for the treatment and prevention of obesity.

[0003] Diabetes mellitus is the most common metabolic disease worldwide. Every day, 1700 new cases of diabetes are diagnosed in the United States, and at least one-third of the 16 million Americans with diabetes are unaware of it. Diabetes is the leading cause of blindness, renal failure, and lower limb amputations in adults and is a major risk factor for cardiovascular disease and stroke.

[0004] Normal glucose homeostasis requires the finely tuned orchestration of insulin secretion by pancreatic beta cells in response to subtle changes in blood glucose levels, delicately balanced with secretion of counter-regulatory hormones such as glucagon. One of the fundamental actions of insulin is to stimulate uptake of glucose from the blood into tissues, especially muscle and fat. Type 1 diabetes results from autoimmune destruction of pancreatic beta cells causing insulin deficiency. Type 2 or non-insulin-dependent diabetes mellitus (NIDDM) accounts for >90% of cases and is characterized by a triad of (1) resistance to insulin action on glucose uptake in peripheral tissues, especially skeletal muscle and adipocytes, (2) impaired insulin action to inhibit hepatic glucose production, and

(3) misregulated insulin secretion (DeFronzo, (1997) *Diabetes Rev.* 5:177-269). In most cases, type 2 diabetes is a polygenic disease with complex inheritance patterns (reviewed in Kahn *et al.*, (1996) *Annu. Rev. Med.* 47:509-531).

[0005] Environmental factors, especially diet, physical activity, and age, interact with genetic predisposition to affect disease prevalence. Susceptibility to both insulin resistance and insulin secretory defects appears to be genetically determined (Kahn, *et al.*). Defects in insulin action precede the overt disease and are seen in non-diabetic relatives of diabetic subjects. In spite of intense investigation, the genes responsible for the common forms of Type 2 diabetes remain unknown.

### **Description of the Invention**

[0006] The present invention is based, at least in part, on the discovery that pro-inflammatory mediators C3aR and C5aR anaphylatoxin receptor transcripts are expressed at high levels in normal mouse and human adipose tissue, more specifically within the white adipose. Additionally, C3aR and C5aR are significantly upregulated in adipose tissue in both genetic and diet induced animal obesity and diabetes models. In a preferred embodiment, the anaphylotoxin receptor molecules of the present invention are capable of recruiting and activating immune cells in white adipose tissue in obese state. These immune cells, predominantly macrophages can release proinflammatory peptides such as TNF $\alpha$  to negatively impact insulin signaling by activating IKK $\beta$ , and JNK1 pathways. Accordingly, the present invention provides methods and compositions for the diagnosis and treatment of metabolic disorders, *e.g.*, obesity, diabetes, and insulin resistance.

[0007] Additionally, the invention relates to the discovery that markers of acute and chronic inflammation osteopontin (OPN), macrophage chemotactic protein-1 (MCP-1) and haptoglobin (HAP) are upregulated in adipose tissue in both genetic and diet induced animal obesity models over time. Still further, the upregulation over time correlated with insulin resistance over time, and is reduced upon treatment with insulin resistance therapy. Thus, the present invention provides methods and compositions for the diagnosis, prognosis, and monitoring of insulin resistance and diabetes.

[0008] In one aspect, the invention provides methods for identifying a nucleic acid or a polypeptide associated with a metabolic disorder, *e.g.*, obesity, diabetes, and insulin resistance. The method includes contacting a sample with a nucleic acid or compound capable of detecting the presence of any one of: an anaphylatoxin receptor (*e.g.*, C3aR,

C5aR) nucleic acid or an anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide; a haptoglobin nucleic acid or haptoglobin polypeptide; an osteopontin nucleic acid or osteopontin polypeptide; or an MCP-1 nucleic acid or MCP-1 polypeptide. When the nucleic acid or compound capable of detecting any one of: anaphylatoxin receptor (e.g., C3aR, C5aR) nucleic acid or polypeptide; haptoglobin nucleic acid or polypeptide; osteopontin nucleic acid or polypeptide; or MCP-1 nucleic acid or polypeptide does detect the presence of any one of the above identified nucleic acid or polypeptides, a nucleic acid or polypeptide associated with a metabolic disorder is identified. Compounds can be designed which are capable of detecting anaphylatoxin receptor (e.g., C3aR, C5aR) nucleic acid or polypeptide; haptoglobin nucleic acid or polypeptide; osteopontin nucleic acid or polypeptide; or MCP-1 nucleic acid or polypeptide. For example, nucleic acids can be detected using probes or amplification methods using designed probes and primers specific for sequences of anaphylatoxin receptor (e.g., C3aR, C5aR) nucleic acid; a haptoglobin nucleic acid; an osteopontin nucleic acid; or an MCP-1 nucleic acid. Additionally, proteins can be detected using peptides, antibodies or compounds which bind to any one of anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide; haptoglobin polypeptide; osteopontin polypeptide; or MCP-1 polypeptide.

[0009] In another aspect, the invention provides methods for identifying a compound capable of treating a metabolic disorder, e.g., obesity, or diabetes. The method includes assaying the ability of the compound to modulate anaphylatoxin receptor (e.g., C3aR, C5aR) nucleic acid expression or anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide activity. In one embodiment, the ability of the compound to modulate anaphylatoxin receptor (e.g., C3aR, C5aR) nucleic acid expression or anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide activity is determined by detecting modulation of inflammatory signaling (e.g., TNF $\alpha$ , IKK, JNK1 signaling). In another embodiment, the ability of the compound to modulate anaphylatoxin receptor (e.g., C3aR, C5aR) nucleic acid expression or anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide activity is determined by detecting modulation of insulin sensitivity. In still another embodiment, the ability of the compound to modulate anaphylatoxin receptor (e.g., C3aR, C5aR) nucleic acid expression or anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide activity is determined by detecting modulation of production of secreted inflammatory proteins (e.g., OPN, MCP-1, HAP). In yet another aspect, the method includes contacting a cell expressing a anaphylatoxin receptor (e.g., C3aR, C5aR) nucleic acid or polypeptide with a test compound and assaying

the ability of the test compound to modulate the expression of a anaphylatoxin receptor (e.g., C3aR, C5aR) nucleic acid or the activity of a anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide.

**[0010]** In another aspect, the invention provides methods for identifying a compound capable of modulating an adipose tissue activity, e.g., insulin sensitivity or obesity. The method includes contacting a cell capable of expressing an anaphylatoxin receptor (e.g., C3aR, C5aR) nucleic acid or polypeptide with a test compound and assaying the ability of the test compound to modulate the expression of an anaphylatoxin receptor (e.g., C3aR, C5aR) nucleic acid or the activity of an anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide.

**[0011]** In another aspect, the invention provides methods for modulating an adipose tissue activity, e.g., insulin sensitivity or obesity. The method includes contacting a composition comprising adipose tissue capable of expressing an anaphylatoxin receptor with an anaphylatoxin receptor (e.g., C3aR, C5aR) modulator, for example, an anti-anaphylatoxin receptor (e.g., C3aR, C5aR) antibody, an anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4 or a fragment thereof, an anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide comprising an amino acid sequence which is at least 90 percent identical to the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, an isolated naturally occurring allelic variant of a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, a small molecule, an antisense anaphylatoxin receptor (e.g., C3aR, C5aR) nucleic acid molecule, a nucleic acid molecule of SEQ ID NO:1 or SEQ ID NO:3, or a fragment thereof, or a ribozyme.

**[0012]** In yet another aspect, the invention features a method for identifying a subject having a metabolic disorder, e.g., obesity, or diabetes. The method includes contacting a sample obtained from the subject, comprising nucleic acid or polypeptide with a compound, and assaying the ability of the test compound to detect any one of anaphylatoxin receptor (e.g., C3aR, C5aR), haptoglobin (HAP), osteopontin (OPN), or MCP-1 nucleic acid; or anaphylatoxin receptor (e.g., C3aR, C5aR), haptoglobin (HAP), osteopontin (OPN), or monocyte chemoattractant protein 1 (MCP-1) polypeptide. When the compound capable of detecting any one of: anaphylatoxin receptor (e.g., C3aR, C5aR) nucleic acid or polypeptide; haptoglobin nucleic acid or polypeptide; osteopontin nucleic acid or polypeptide; or monocyte chemoattractant protein 1 nucleic acid or polypeptide does detect the presence of

any one of the above identified nucleic acid or polypeptides, a subject having a metabolic disorder, or at risk of developing a metabolic disorder is identified. Compounds can be designed which are capable of detecting anaphylatoxin receptor (e.g., C3aR, C5aR) nucleic acid or polypeptide; haptoglobin nucleic acid or polypeptide; osteopontin nucleic acid or polypeptide; or MCP-1 nucleic acid or polypeptide. For example, nucleic acids can be detected using probes or amplification methods using designed probes and primers specific for sequences of anaphylatoxin receptor (e.g., C3aR, C5aR) nucleic acid; a haptoglobin nucleic acid; an osteopontin nucleic acid; or an MCP-1 nucleic acid. Additionally, proteins can be detected using peptides, antibodies or compounds which bind to any one of anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide; haptoglobin polypeptide; osteopontin polypeptide; or MCP-1 polypeptide. Such methods are useful for identification of a subject having a metabolic disorder, or at risk of developing a metabolic disorder selected from obesity, diabetes, or insulin resistance.

[0013] In yet another aspect, the invention features a method for treating a subject having a metabolic disorder, *e.g.*, obesity, or diabetes, characterized by aberrant anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide activity or aberrant anaphylatoxin receptor (e.g., C3aR, C5aR) nucleic acid expression. The method includes administering to the subject an anaphylatoxin receptor (e.g., C3aR, C5aR) modulator, *e.g.*, in a pharmaceutically acceptable formulation or by using a gene therapy vector. Embodiments of this aspect of the invention include the anaphylatoxin receptor (e.g., C3aR, C5aR) modulator being a small molecule, an anti-anaphylatoxin receptor (e.g., C3aR, C5aR) antibody, an anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide, *e.g.*, comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4 or a fragment thereof, an anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide comprising an amino acid sequence which is at least 90 percent identical to the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, an isolated naturally occurring allelic variant of a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, an antisense anaphylatoxin receptor (e.g., C3aR, C5aR) nucleic acid molecule, a nucleic acid molecule of SEQ ID NO:1 or SEQ ID NO:3 or a fragment thereof, or a ribozyme.

[0014] Other features and advantages of the invention will be apparent from the following description and claims.

**[0015]** The invention provides methods and compositions for the diagnosis and treatment of a metabolic disorder, *e.g.*, obesity, or diabetes. The invention is based, at least in part, on the discovery that anaphylatoxin receptor (C3aR and C5aR) transcripts are expressed at high levels in adipose tissue. Furthermore, upregulated expression of the anaphylatoxin receptor (C3aR and C5aR) transcripts are also seen in the adipose of genetic (*ob/ob*, *db/db*) or diet induced obese mice. In one embodiment, the anaphylatoxin receptor (C3aR and C5aR) molecules modulate the activity of one or more proteins involved in the insulin signaling pathway, (*e.g.*, IKK $\beta$ , NF- $\kappa$ B, JNK), resulting in modulating signaling pathway involved in regulation of metabolic functioning. In a preferred embodiment, the anaphylatoxin receptor (C3aR and C5aR) molecules of the present invention are capable of modulating the production and activity of peptides involved in signaling pathway involved in regulation of metabolic and insulin signaling to thereby modulate the effects of insulin sensitivity and glucose homeostasis.

**[0016]** The nucleotide sequence of human anaphylatoxin receptors C3aR, C5aR (GenBank Accession Nos. Z73157 and X57250) are depicted in SEQ ID NO:1 and SEQ ID NO:3, respectively. The amino acid sequence corresponds to SEQ ID NO:2 and SEQ ID NO:4.

**[0017]** The nucleotide sequence of murine anaphylatoxin receptors C3aR, and C5aR (GenBank Accession Nos. U77461 and S46665 L05630) are depicted in SEQ ID NO:9 and SEQ ID NO:11, respectively. The amino acid sequence corresponds to SEQ ID NO:10 and SEQ ID NO:12.

**[0018]** The nucleotide sequence of human haptoglobin (HAP) (GenBank Accession No. NM005143) is depicted in SEQ ID NO:5. The amino acid sequence corresponds to SEQ ID NO:6.

**[0019]** The nucleotide sequence of murine haptoglobin (HAP) (GenBank Accession No. NM017370) is depicted in SEQ ID NO:13. The amino acid sequence corresponds to SEQ ID NO:14.

**[0020]** The nucleotide sequence of human osteopontin (OPN) (GenBank Accession No. J04765) is depicted in SEQ ID NO:7. The amino acid sequence corresponds to SEQ ID NO:8.

**[0021]** The nucleotide sequence of murine osteopontin (OPN) (GenBank Accession No. AF515708) is depicted in SEQ ID NO:15. The amino acid sequence corresponds to SEQ ID NO:16.

[0022] The nucleotide sequence of human monocyte chemoattractant protein 1 (MCP-1) (GenBank Accession No. X14768) are depicted in SEQ ID NO:17. The amino acid sequence corresponds to SEQ ID NO:18.

[0023] The nucleotide sequence of murine monocyte chemoattractant protein 1 (MCP-1) (GenBank Accession No. L13763) are depicted in SEQ ID NO:19. The amino acid sequence corresponds to SEQ ID NO:20.

[0024] As used herein, the term "metabolic disorder" includes a disorder, disease or condition which is caused or characterized by an abnormal metabolism (*i.e.*, the chemical changes in living cells by which energy is provided for vital processes and activities) in a subject. Metabolic disorders include diseases, disorders, or conditions associated with hyperglycemia, hypoglycemia, or aberrant adipose cell (*e.g.*, brown or white adipose cell) phenotype or function. Metabolic disorders can be characterized by a misregulation (*e.g.*, an aberrant downregulation or upregulation) of an anaphylatoxin receptor (*e.g.*, C3aR, C5aR) activity. Metabolic disorders can detrimentally affect cellular functions such as cellular proliferation, growth, differentiation, or migration, cellular regulation of homeostasis, inter- or intra-cellular communication; tissue function, such as liver function, skeletal muscle function, or adipocyte function; systemic responses in an organism, such as hormonal responses (*e.g.*, insulin response). Examples of metabolic disorders include obesity, insulin resistance, diabetes, endocrine abnormalities, triglyceride storage disease, Bardet-Biedl syndrome, Lawrence-Moon syndrome, Prader-Labhart-Willi syndrome, and disorders of lipid metabolism.

[0025] Obesity is defined as a body mass index (BMI) of 30 kg/m<sup>2</sup> or more (National Institute of Health, Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults (1998)). However, the invention is also intended to include a disease, disorder, or condition that is characterized by a body mass index (BMI) of 25 kg/m<sup>2</sup> or more, 26 kg/m<sup>2</sup> or more, 27 kg/m<sup>2</sup> or more, 28 kg/m<sup>2</sup> or more, 29 kg/m<sup>2</sup> or more, 29.5 kg/m<sup>2</sup> or more, or 29.9 kg/m<sup>2</sup> or more, all of which are typically referred to as overweight (National Institute of Health, Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults (1998)).

[0026] "Subject", as used herein, can refer to a mammal, *e.g.*, a human, or to an experimental or naturally occurring animal disease model. The subject can also be a non-human animal, *e.g.*, a horse, cow, goat, or other domestic animal. A subject, *e.g.*, a human

subject, can also be a patient, *i.e.*, an individual receiving medical attention, care, or treatment.

**[0027]** As used interchangeably herein, an "anaphylatoxin receptor (e.g., C3aR, C5aR) activity," "biological activity of anaphylatoxin receptor" or "functional activity of anaphylatoxin receptor," includes an activity exerted by an anaphylatoxin receptor (e.g., C3aR, C5aR) protein, polypeptide or nucleic acid molecule on an anaphylatoxin receptor (e.g., C3aR, C5aR) responsive cell or tissue, or on an anaphylatoxin receptor (e.g., C3aR, C5aR) protein ligand, *e.g.*, an C3a, C5a, as determined *in vivo*, or *in vitro*, according to standard techniques. An anaphylatoxin receptor (e.g., C3aR, C5aR) activity can be a direct activity, such as an association with an anaphylatoxin (e.g., C3a, C5a) target molecule. As used herein, a "substrate" or "target molecule" or "binding partner" is a molecule with which an anaphylatoxin receptor (e.g., C3aR, C5aR) protein binds or interacts in nature, such that an anaphylatoxin receptor (e.g., C3aR, C5aR) mediated function, *e.g.*, modulation of a inflammatory signaling, is achieved. An anaphylatoxin receptor (e.g., C3aR, C5aR) target molecule can be a non-anaphylatoxin receptor (e.g., C3aR, C5aR) molecule (*e.g.*, a protein, a metal ion) or an anaphylatoxin receptor (e.g., C3aR, C5aR) protein or polypeptide. Examples of such target molecules include proteins in the same metabolic pathway as the anaphylatoxin receptor (e.g., C3aR, C5aR) protein, *e.g.*, proteins which may function upstream (including both stimulators and inhibitors of activity) or downstream of the anaphylatoxin receptor (e.g., C3aR, C5aR) protein in a pathway involving regulation of metabolism. Alternatively, an anaphylatoxin receptor (e.g., C3aR, C5aR) activity is an indirect activity, such as a cellular signaling activity mediated as a consequence of the interaction of the anaphylatoxin receptor (e.g., C3aR, C5aR) protein with an anaphylatoxin receptor (e.g., C3aR, C5aR) target molecule or substrate.

**[0028]** The biological activities of anaphylatoxin receptor (e.g., C3aR, C5aR) are described herein. For example, the anaphylatoxin receptor (e.g., C3aR, C5aR) proteins can have one or more of the following activities: (1) the ability to interact with a non-anaphylatoxin receptor (e.g., C3aR, C5aR) molecule (e.g., C3a, C5a) within or on the surface of the same cell which expresses it; (2) the ability to interact with a non-anaphylatoxin receptor (e.g., C3aR, C5aR) molecule (e.g., C3a, C5a) within or on the surface of a different cell; (3) the ability to activate anaphylatoxin receptor-independent signal transduction pathway (*e.g.*, TNF, JNK, IKK $\beta$ ) through activating macrophages to release pro-inflammatory cytokines, including but not limited to, TNF $\alpha$ ; (4) the ability to

modulate C3a or C5a gene expression or protein activity; (5) the ability to modulate insulin signaling (6) the ability to modulate glucose metabolism, *e.g.*, glucose secretion or uptake; or (7) the ability to modulate insulin metabolism, *e.g.*, insulin secretion or uptake. Thus, the anaphylatoxin receptor (*e.g.*, C3aR, C5aR) proteins can be used to, for example, (1) modulate the interaction with a non-anaphylatoxin receptor (*e.g.*, C3aR, C5aR) molecule within or on the surface of the same cell which expresses it; (2) modulate the interaction with a non-anaphylatoxin receptor (*e.g.*, C3aR, C5aR) molecule within or on the surface of a different cell; (3) activate an anaphylatoxin receptor-dependent signal transduction pathway; (4) modulate C3a or C5a gene expression or protein activity; (5) modulate an insulin signaling response; (6) modulate glucose metabolism, *e.g.*, glucose secretion or uptake; or (7) modulate insulin metabolism, *e.g.*, insulin secretion or metabolism.

[0029] As used herein, "metabolic activity" may include an activity exerted by a cell, *e.g.*, an adipose cell such as for example a white fat adipose cell, or an activity that takes place in an adipose cell. For example, such activities include cellular processes that contribute to the physiological role of adipose tissue (whether directly or indirectly, *e.g.*, through signaling), in regulation of metabolism and include, but are not limited to, cell proliferation, differentiation, growth, migration, programmed cell death, uncoupled mitochondrial respiration, thermogenesis, and insulin sensitivity.

[0030] Various aspects of the invention are described in further detail in the following subsections.

### Screening Assays

[0031] The invention provides methods (also referred to herein as a "screening assays") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules or other drugs) which bind to anaphylatoxin receptor (*e.g.*, C3aR, C5aR) polypeptides, have a stimulatory or inhibitory effect on, for example, anaphylatoxin receptor (*e.g.*, C3aR, C5aR) expression or anaphylatoxin receptor (*e.g.*, C3aR, C5aR) activity, or have a stimulatory or inhibitory effect on, for example, the expression or activity of an anaphylatoxin receptor (*e.g.*, C3aR, C5aR) substrate.

[0032] In one embodiment, the invention provides assays for screening candidate or test compounds which are substrates of an anaphylatoxin receptor (*e.g.*, C3aR, C5aR) polypeptide or polypeptide, or biologically active portion thereof. In another embodiment, the invention provides assays for screening candidate or test compounds which bind to or

modulate the activity of an anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide, or biologically active portion thereof. The test compounds of the invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; 'one-bead one-compound' library methods; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer, and small molecule libraries of compounds (Lam, K.S. (1997) *Anticancer Drug Des.* 12:145).

**[0033]** Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909; Erb *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann *et al.* (1994). *J. Med. Chem.* 37:2678; Cho *et al.* (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and in Gallop *et al.* (1994) *J. Med. Chem.* 37:1233.

**[0034]** Libraries of compounds may be presented in solution (e.g., Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (U.S. Patent No. 5,223,409), spores (U.S. Patent No. 5,223,409), plasmids (Cull *et al.* (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or phage (Scott and Smith (1990) *Science* 249:386-390; Devlin (1990) *Science* 249:404-406; Cwirla *et al.* (1990) *Proc. Natl. Acad. Sci.* 87:6378-6382; Felici (1991) *J. Mol. Biol.* 222:301-310); Ladner *supra*).

**[0035]** In one embodiment, an assay is a cell-based assay in which a cell expressing an anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide, or biologically active portion thereof, is contacted with a test compound and the ability of the test compound to modulate anaphylatoxin receptor (e.g., C3aR, C5aR) activity is determined. Determining the ability of the test compound to modulate anaphylatoxin receptor (e.g., C3aR, C5aR) activity can be accomplished by monitoring, for example, modulation of the serum level of OPN, MCP-1 or HAP.

**[0036]** In an embodiment, an assay is a cell-based assay in which a cell which expresses a constitutively active anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide, or a constitutively active portion thereof, is contacted with a test compound and the ability of the test compound to inhibit anaphylatoxin receptor (e.g., C3aR, C5aR) activity is determined.

**[0037]** In one embodiment, an assay is a cell-based assay in which a cell, which expresses a constitutively active anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide, or a constitutively active portion thereof, is contacted with a test compound, and the ability of the test compound to modulate insulin signaling (eg., NF- $\kappa$ B, IKK $\beta$ , JNK1 signaling) is determined.

**[0038]** The ability of the test compound to modulate anaphylatoxin receptor (e.g., C3aR, C5aR) binding to a substrate, *e.g.*, an anaphylatoxin (eg., C3a, C5a) protein, or to bind anaphylatoxin receptor (e.g., C3aR, C5aR) itself can also be determined. Determining the ability of the test compound to modulate anaphylatoxin receptor (e.g., C3aR, C5aR) binding to a substrate can be accomplished, for example, by coupling the anaphylatoxin receptor (e.g., C3aR, C5aR) ligand, *e.g.*, an anaphylatoxin (eg., C3a, C5a) protein, with a radioisotope, an enzymatic label, or a fluorescent label such that binding of the anaphylatoxin receptor (e.g., C3aR, C5aR) substrate to anaphylatoxin receptor (e.g., C3aR, C5aR) can be determined by detecting the labeled anaphylatoxin receptor (e.g., C3aR, C5aR) substrate in a complex. Alternatively, anaphylatoxin receptor (e.g., C3aR, C5aR) can be coupled with a radioisotope, an enzymatic label, or a fluorescent label to monitor the ability of a test compound to modulate anaphylatoxin receptor (e.g., C3aR, C5aR) binding to a anaphylatoxin receptor (e.g., C3aR, C5aR) substrate in a complex. Determining the ability of the test compound to bind anaphylatoxin receptor (e.g., C3aR, C5aR) can be accomplished, for example, by coupling the compound with a radioisotope, an enzymatic label, or a fluorescent label such that binding of the compound to anaphylatoxin receptor (e.g., C3aR, C5aR) can be determined by detecting the labeled anaphylatoxin receptor (e.g., C3aR, C5aR) compound in a complex. For example, compounds (*e.g.*, anaphylatoxin receptor (e.g., C3aR, C5aR) substrates) can be labeled with  $^{125}$ I,  $^{35}$ S,  $^{14}$ C, or  $^3$ H, either directly or indirectly, and the radioisotope detected by direct counting of radioemmission or by scintillation counting. Alternatively, compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product. Compounds can be fluorescently labeled with, for example, fluorescein, rhodamine, AMCA, or TRF, and the fluorescent label detected by exposure of the compound to a specific wavelength of light.

**[0039]** It is also within the scope of this invention to determine the ability of a compound (*e.g.*, a anaphylatoxin receptor (e.g., C3aR, C5aR) substrate, *e.g.*, an

anaphylatoxin (e.g., C3a, C5a)) to interact with anaphylatoxin receptor (e.g., C3aR, C5aR) without the labeling of any of the interactants. For example, a microphysiometer can be used to detect the interaction of a compound with anaphylatoxin receptor (e.g., C3aR, C5aR) without the labeling of either the compound or anaphylatoxin receptor. (See McConnell, H. M. *et al.* (1992) *Science* 257:1906-1912.) As used herein, a "microphysiometer" (e.g., the Cytosensor® Microphysiometer System by Molecular Devices Corp., Sunnyvale CA) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a compound and anaphylatoxin receptor.

[0040] In another embodiment, an assay is a cell-based assay comprising contacting a cell which expresses a anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide or anaphylatoxin receptor (e.g., C3aR, C5aR) target molecule (e.g., a anaphylatoxin receptor (e.g., C3aR, C5aR) ligand, e.g., an anaphylatoxin (eg., C3a, C5a) protein) with a test compound and determining the ability of the test compound to modulate (e.g., stimulate or inhibit) the activity of the anaphylatoxin receptor (e.g., C3aR, C5aR) target molecule. Determining the ability of the test compound to modulate the activity of an anaphylatoxin receptor (e.g., C3aR, C5aR) target molecule can be accomplished, for example, by determining the ability of the anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide to bind to or interact with the anaphylatoxin receptor (e.g., C3aR, C5aR) target molecule in the presence of the test compound, or by determining the ability of the anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide to bind to or interact with the anaphylatoxin receptor (e.g., C3aR, C5aR) target molecule before or after exposure of the anaphylatoxin receptor (e.g., C3aR, C5aR) target molecule with the test compound.

[0041] Determining the ability of the anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide, or a biologically active fragment thereof, to bind to or interact with an anaphylatoxin receptor (e.g., C3aR, C5aR) target molecule can be accomplished by any one of the methods described above for determining direct binding. In a preferred embodiment, determining the ability of the anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide to bind or interact with an anaphylatoxin receptor (e.g., C3aR, C5aR) target molecule, e.g., an anaphylatoxin (eg, C3a, C5a) protein, can be accomplished by determining a change in the biological or chemical activity of the resulting from the binding or interaction of the anaphylatoxin receptor (e.g., C3aR, C5aR) target molecule with the anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide. For example, the activity of the target molecule can be

determined by detecting an enzymatic or catalytic activity of the target using an appropriate substrate, by detecting the induction of a reporter gene (comprising a target-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or by detecting a target-regulated cellular response.

**[0042]** In yet another embodiment, an assay of the invention is a cell-free assay in which an anaphylatoxin receptor (*e.g.*, C3aR, C5aR) polypeptide, or biologically active portion thereof, is contacted with a test compound and the ability of the test compound to bind to the anaphylatoxin receptor (*e.g.*, C3aR, C5aR) polypeptide, or biologically active portion thereof, is determined. Preferred biologically active portions of the anaphylatoxin receptor (*e.g.*, C3aR, C5aR) polypeptides to be used in any of the assays of the invention include fragments which participate in interactions with non-anaphylatoxin receptor (*e.g.*, C3aR, C5aR) molecules, *e.g.*, fragments with high surface probability scores. Binding of the test compound to the anaphylatoxin receptor (*e.g.*, C3aR, C5aR) polypeptide can be determined either directly or indirectly as described above. In a preferred embodiment, the assay includes contacting the anaphylatoxin receptor (*e.g.*, C3aR, C5aR) polypeptide, or biologically active portion thereof, with a known compound which binds anaphylatoxin receptor (*e.g.*, C3aR, C5aR) to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with an anaphylatoxin receptor (*e.g.*, C3aR, C5aR) polypeptide, wherein determining the ability of the test compound to interact with an anaphylatoxin receptor (*e.g.*, C3aR, C5aR) polypeptide comprises determining the ability of the test compound to preferentially bind to anaphylatoxin receptor (*e.g.*, C3aR or C5aR), or biologically active portion thereof, as compared to the known compound.

**[0043]** In another embodiment, the assay is a cell-free assay in which an anaphylatoxin receptor (*e.g.*, C3aR, C5aR) polypeptide, or biologically active portion thereof, is contacted with a test compound and the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the anaphylatoxin receptor (*e.g.*, C3aR, C5aR) polypeptide, or biologically active portion thereof, is determined. Determining the ability of the test compound to modulate the activity of an anaphylatoxin receptor (*e.g.*, C3aR, C5aR) polypeptide can be accomplished, for example, by determining the ability of the anaphylatoxin receptor (*e.g.*, C3aR, C5aR) polypeptide to bind to or interact with a anaphylatoxin receptor (*e.g.*, C3aR, C5aR) target molecule by any of the methods described above for determining direct binding. Determining the ability of the anaphylatoxin receptor

(e.g., C3aR, C5aR) polypeptide to bind to an anaphylatoxin receptor (e.g., C3aR, C5aR) target molecule can also be accomplished using a technology such as real-time Biomolecular Interaction Analysis (BIA). (See, e.g., Sjolander, S. and Urbaniczky, C. (1991) *Anal. Chem.* 63:2338-2345 and Szabo *et al.* (1995) *Curr. Opin. Struct. Biol.* 5:699-705.) As used herein, "BIA" is a technology for studying biospecific interactions in real time, without labeling any of the interactants (e.g., BIACore). Changes in the optical phenomenon of surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

[0044] In an alternative embodiment, determining the ability of the test compound to modulate the activity of an anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide can be accomplished by determining the ability of the anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide to further modulate the activity of a downstream or upstream effector of an anaphylatoxin receptor (e.g., C3aR, C5aR) target molecule. For example, the activity of the effector molecule on an appropriate target can be determined or the binding of the effector to an appropriate target can be determined, as previously described.

[0045] In yet another embodiment, determining the ability of the test compound to modulate the activity of an anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide can be accomplished by determining the ability of the test compound to modulate the activity of an anaphylatoxin receptor (e.g., C3aR, C5aR) target molecule, e.g., an anaphylatoxin receptor (e.g., C3aR, C5aR) ligans, e.g., an anaphylatoxin (eg, C3a, C5a) protein. In a preferred embodiment, the assay includes contacting the anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide, or biologically active portion thereof, with a known compound which binds anaphylatoxin receptor, e.g., an anaphylatoxin receptor ligand (e.g., C3a, C5a), to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with the known compound, wherein determining the ability of the test compound to interact with the known compound includes determining the ability of the test compound to preferentially bind to the known compound, or biologically active portion thereof, as compared to the anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide.

[0046] In yet another embodiment, the cell-free assay involves contacting an anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide, or biologically active portion thereof, with a known compound which binds the anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide to form an assay mixture, contacting the assay mixture with a test compound,

and determining the ability of the test compound to interact with the anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide, wherein determining the ability of the test compound to interact with the anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide comprises determining the ability of the anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide to preferentially bind to or modulate the activity of an anaphylatoxin receptor (e.g., C3aR, C5aR) target molecule as compared to the known compound.

[0047] In one or more embodiments of the above assay methods of the invention, it may be desirable to immobilize either anaphylatoxin receptor (e.g., C3aR, C5aR) or its target molecule to facilitate separation of complexed from uncomplexed forms of anaphylatoxin receptor (e.g., C3aR, C5aR) and its target molecule, as well as to accommodate automation of the assay. Binding of a test compound to an anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide, or interaction of an anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase/anaphylatoxin receptor (e.g., C3aR, C5aR) fusion proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized micrometer plates, which can then combined with the test compound or the test compound and either the non-adsorbed target protein or anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide, and the mixture incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the beads or micrometer plates are washed to remove any unbound components, the matrix immobilized in the case of beads, and the presence of complex is then determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of anaphylatoxin receptor (e.g., C3aR, C5aR) binding or activity determined using standard techniques.

[0048] Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either an anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide or an anaphylatoxin receptor (e.g., C3aR, C5aR) target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide or target molecules can be prepared

from biotin-NHS (N-hydroxy-succinimide) using techniques known in the art (e.g., a biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96-well microtiter plates (Pierce Chemicals). Alternatively, antibodies reactive with anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide or its target molecules, but which do not interfere with binding of the anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide to its target molecule can be derivatized to the wells of the plate, such that complexes of target bound to anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide will be trapped in the wells by the antibody. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide or target molecule.

**[0049]** In another embodiment, modulators of anaphylatoxin receptor (e.g., C3aR, C5aR) expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of anaphylatoxin receptor (e.g., C3aR, C5aR) mRNA or polypeptide in the cell is determined. The level of expression of anaphylatoxin receptor (e.g., C3aR, C5aR) mRNA or polypeptide in the presence of the candidate compound is compared to the level of expression of anaphylatoxin receptor (e.g., C3aR, C5aR) mRNA or polypeptide in the absence of the candidate compound. The candidate compound can then be identified as a modulator of anaphylatoxin receptor (e.g., C3aR, C5aR) expression based on this comparison. For example, when expression of anaphylatoxin receptor (e.g., C3aR, C5aR) mRNA or polypeptide is greater (statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of anaphylatoxin receptor (e.g., C3aR, C5aR) mRNA or polypeptide expression. Alternatively, when expression of anaphylatoxin receptor (e.g., C3aR, C5aR) mRNA or polypeptide is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of anaphylatoxin receptor (e.g., C3aR, C5aR) mRNA or polypeptide expression. The level of anaphylatoxin receptor (e.g., C3aR, C5aR) mRNA or polypeptide expression in the cells can be determined by methods described herein for detecting anaphylatoxin receptor (e.g., C3aR, C5aR) mRNA or polypeptide.

**[0050]** In yet another aspect of the invention, the anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptides can be used as "bait proteins" in a two-hybrid assay or three-hybrid assay (see, e.g., U.S. Patent No. 5,283,317; Zervos *et al.* (1993) *Cell* 72:223-232; Madura *et*

*al.* (1993) *J. Biol. Chem.* 268:12046-12054; Bartel *et al.* (1993) *Biotechniques* 14:920-924; Iwabuchi *et al.* (1993) *Oncogene* 8:1693-1696; and Brent WO 94/10300), to identify other proteins which bind to or interact with anaphylatoxin receptor (e.g., C3aR, C5aR) (e.g., "anaphylatoxin receptor-binding proteins" or "anaphylatoxin receptor-bp") and are involved in anaphylatoxin receptor (e.g., C3aR, C5aR) activity. Such anaphylatoxin receptor-binding proteins are also likely to be involved in the propagation of pathway signals mediated by the anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptides or anaphylatoxin receptor (e.g., C3aR, C5aR) targets as, for example, upstream or downstream elements of an anaphylatoxin receptor-mediated signaling pathway. If there is an enhancement or stimulation of an anaphylatoxin receptor-mediated signaling pathway, the anaphylatoxin receptor-binding proteins are likely to be anaphylatoxin receptor (e.g., C3aR, C5aR) stimulators.

Alternatively, if there is a reduction of an anaphylatoxin receptor-mediated signaling pathway, the anaphylatoxin receptor-binding proteins are likely to be anaphylatoxin receptor (e.g., C3aR, C5aR) inhibitors.

[0051] The two-hybrid, or "bait and prey", system is based on the modular nature of most transcription factors which consist of separable DNA-binding and activation domains. This enables an assay that utilizes two different DNA constructs. Briefly, one construct containing a gene sequence that encodes an anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide ("bait protein") is fused to a gene encoding the DNA binding domain of a known transcription factor (e.g., GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences that encodes an unidentified protein (i.e., the "prey" or "sample"), is fused to a gene that encodes the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact *in vivo* to form a complex of the anaphylatoxin receptor (e.g., C3aR, C5aR) and the target molecule, the DNA-binding and activation domains of the transcription factor will be brought into close proximity to form a functional transcription factor. A reporter gene (e.g., LacZ) operably linked to a transcriptional regulatory site responsive to the transcription factor will then be transcribed. Detection of the expression of the reporter gene enables the identification and isolation of cell colonies containing the functional transcription factor. Subsequently, these cell colonies can then be used to clone and identify the sequence of the "bait" protein.

[0052] In another aspect, the invention pertains to a combination of two or more of the assays described herein. For example, a modulating agent can be identified using a cell-based or a cell-free assay, and the ability of the agent to modulate the activity of an

anaphylatoxin receptor (e.g., C3aR, C5aR) protein can be confirmed *in vivo*, e.g., in an animal such as an animal model for obesity or diabetes. Examples of animals that can be used include the transgenic mouse described in U.S. Patent No. 5,932,779 that contains a mutation in an endogenous melanocortin-4-receptor (MC4-R) gene; animals having mutations which lead to syndromes that include obesity symptoms (described in, for example, Friedman, J. M. *et al.* (1991) *Mamm. Gen.* 1:130-144; Friedman, J. M. and Liebel, R. L. (1992) *Cell* 69:217-220; Bray, G. A. (1992) *Prog. Brain Res.* 93:333-341; and Bray, G. A. (1989) *Amer. J. Clin. Nutr.* 5:891-902); the mice with a diabetes mutation (db) which is attributed to a mutation in the leptin receptor gene (*Lepr*<sup>db</sup>; described in, for example, Chen, H. *et al.* (1996) *Cell* 84:491-5; Chua SC Jr *et al.* (1996) *Science* 271:994-6; and Lee, G.H. et al (1996) *Nature* 379:632-5); the mice homozygous for the obese (ob) mutation (described in, for example, MacDougald, O.A. *et al* (1995) *Proc. Natl. Acad. Sci. USA* 92:9034-7); the animals described in Stubdal H. *et al.* (2000) *Mol. Cell Biol.* 20(3):878-82 (the mouse tubby phenotype characterized by maturity-onset obesity); the animals described in Abadie J.M. *et al.* *Lipids* (2000) 35(6):613-20 (the obese Zucker rat (ZR), a genetic model of human youth-onset obesity and type 2 diabetes mellitus); the animals described in Shaughnessy S. *et al.* (2000) *Diabetes* 49(6):904-11 (mice null for the adipocyte fatty acid binding protein); the animals described in Loskutoff D.J. *et al.* (2000) *Ann. N. Y. Acad. Sci.* 902:272-81 (the fat mouse); or animals having mutations which lead to syndromes that include diabetes (described in, for example, Alleva *et al.* (2001) *J. Clin. Invest.* 107:173-180; Arakawa *et al.* (2001) *Br. J. Pharmacol.* 132:578-586; Nakamura *et al.* (2001) *Diabetes Res. Clin. Pract.* 51:9-20; O'Harte *et al.* (2001) *Regul. Pept.* 96:95-104; Yamanouchi *et al.* (2000) *Exp. Anim.* 49:259-266; Hoenig *et al.* (2000) *Am. J. Pathol.* 157:2143-2150; Reed *et al.* (2000) *Metabolism* 49:1390-1394; and Clark *et al.* (2000) *J. Pharmacol. Toxicol. Methods* 43:1-10). Other examples of animals that may be used include non-recombinant, non-genetic animal models of obesity such as, for example, rabbit, mouse, or rat models in which the animal has been exposed to either prolonged cold, thereby, inducing hypertrophy of BAT and increasing BAT thermogenesis (Himms-Hagen, J. (1990), *supra*). Alternatively, a non-genetic animal model of obesity, e.g., diet-induced obesity, can be used, e.g., by long-term overfeeding or feeding on a high fat diet.

[0053] In another aspect, the invention pertains to computer modeling and searching technologies to identify compounds, or improve previously identified compounds, that can modulate anaphylatoxin receptor (e.g., C3aR, C5aR) gene expression or protein activity.

Having identified such a compound or composition enables identification of active sites or regions, as well as other sites or regions critical in the function of the protein. Such active sites are often ligand, *e.g.*, substrate, binding sites. The active site can be identified using methods known in the art including, for example, from the amino acid sequences of peptides, from the nucleotide sequences of nucleic acids, or from studies of complexes of the relevant compound or composition with its natural ligand. In the latter case, chemical or X-ray crystallographic methods are useful in identifying residues in the active site by locating the position of the complexed ligand.

**[0054]** The three dimensional geometric structure of the active site can be determined using known methods, including X-ray crystallography, from which spatial details of the molecular structure can be obtained. Additionally, solid or liquid phase NMR can be used to determine certain intramolecular distances. Any other experimental method of structure determination known in the art can be used to obtain partial or complete geometric structures. The geometric structures measured with a complexed ligand, natural or artificial, can increase the accuracy of the active site structure determined.

**[0055]** When only an incomplete or insufficiently accurate structure is determined, methods of computer based numerical modeling can be used to complete or improve the accuracy of the structure. Any recognized modeling method may be used, including parameterized models specific to particular biopolymers, such as proteins or nucleic acids, molecular dynamics models based on computing molecular motions, statistical mechanics models based on thermal ensembles, or combined models. For most types of models, standard molecular force fields, which include the forces between constituent atoms and groups, are necessary, and can be selected from force fields known in physical chemistry. The incomplete or less accurate experimental structures can serve as constraints on the complete and more accurate structures computed by these modeling methods.

**[0056]** Having determined the structure of the active site, either experimentally, by modeling, or by a combination of approaches, candidate modulating compounds can be identified by searching databases containing compounds along with information on their molecular structure. Such searches seek compounds having structures that match the determined active site structure and that interact with the groups defining the active site. Such a search can be manual, but is preferably computer assisted. Compounds identified using these search methods can be tested in any of the screening assays described herein to verify their ability to modulate anaphylatoxin receptor (*e.g.*, C3aR, C5aR) activity.

[0057] Alternatively, these methods can be used to identify improved modulating compounds from an already known modulating compound or ligand. The composition of the known compound can be modified and the structural effects of the modification can be determined by applying the experimental and computer modeling methods described above to the new composition. The altered structure is then compared to the active site structure of the compound to determine if an improved fit or interaction results. In this manner, systematic variations in composition, such as by varying side groups, can be quickly evaluated to obtain modified modulating compounds or ligands with improved specificity or activity.

[0058] Kaul ((1998) *Prog. Drug Res.* 50:9-105) provides a review of modeling techniques for the design of receptor ligands and drugs. Computer programs that screen and graphically depict chemicals are available from companies such as BioDesign, Inc. (Pasadena, CA.), Oxford Molecular Design (Oxford, UK), and Hypercube, Inc. (Cambridge, Ontario).

[0059] Although described above with reference to design and generation of compounds which can alter the ability of anaphylatoxin receptor (e.g., C3aR, C5aR) to bind its target molecule, *e.g.*, a substrate, one can also screen libraries of known compounds, including natural products or synthetic chemicals, and biologically active materials, including proteins, for compounds which are inhibitors or activators.

[0060] This invention further pertains to novel agents identified by the above-described screening assays. Accordingly, it is within the scope of this invention to further use an agent identified as described herein in an appropriate animal model, *e.g.*, animal models for obesity or diabetes.

[0061] In addition, transgenic animals that express a human anaphylatoxin receptor (e.g., C3aR, C5aR) can be used to confirm the *in vivo* effects of a modulator of anaphylatoxin receptor (e.g., C3aR, C5aR) identified by a cell-based or cell-free screening assay described herein. Animals of any non-human species, including, but not limited to, mice, rats, rabbits, guinea pigs, pigs, micro-pigs, goats, and non-human primates, *e.g.*, baboons, monkeys, and chimpanzees, may be used to generate anaphylatoxin receptor (e.g., C3aR, C5aR) transgenic animals. Alternatively, the transgenic animal comprises a cell, or cells, that includes a gene which misexpresses an endogenous anaphylatoxin receptor (e.g., C3aR, C5aR) orthologue such that expression is disrupted, *e.g.*, a knockout animal. Such

animals are also useful as a model for studying the disorders which are related to mutated or misexpressed anaphylatoxin receptor (e.g., C3aR, C5aR) alleles.

[0062] Any technique known in the art may be used to introduce the human anaphylatoxin receptor (e.g., C3aR, C5aR) transgene into non-human animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to, pronuclear microinjection (U.S. Patent No. 4,873,191); retrovirus mediated gene transfer into germ lines (Van der Putten *et al.* (1985) *Proc. Natl. Acad. Sci. USA* 82:6148-6152); gene targeting in embryonic stem cells (Thompson *et al.* (1989) *Cell* 56:313-321); electroporation of embryos (Lo (1983) *Mol Cell. Biol.* 3:1803-1814); and sperm-mediated gene transfer (Lavitrano *et al.* (1989) *Cell* 57:717-723). For a review of such techniques, see Gordon (1989) Transgenic Animals, *Intl. Rev. Cytol.* 115:171-229, which is incorporated by reference herein in its entirety.

[0063] The invention provides for transgenic animals that carry the anaphylatoxin receptor (e.g., C3aR, C5aR) transgene in all their cells, as well as animals which carry the transgene in some, but not all their cells, i.e., mosaic animals. The transgene may be integrated as a single transgene or in concatamers, *e.g.*, head-to-head tandems or head-to-tail tandems. The transgene may also be selectively introduced into and activated in a particular cell type by following, for example, the teaching of Lasko *et al.* ((1992) *Proc. Natl. Acad. Sci. USA* 89: 6232-6236). The regulatory sequences required for such a cell-type specific activation will depend upon the particular cell type of interest and will be apparent to those of skill in the art. When it is desired that the anaphylatoxin receptor (e.g., C3aR, C5aR) transgene be integrated into the chromosomal site of the endogenous anaphylatoxin receptor (e.g., C3aR, C5aR) gene, gene targeting is preferred. Briefly, this technique employs vectors that contain nucleotide sequences homologous to the endogenous anaphylatoxin receptor (e.g., C3aR, C5aR) gene and/or sequences flanking the gene. The vectors are designed to integrate into the chromosomal site of the endogenous anaphylatoxin receptor (e.g., C3aR, C5aR) gene, thereby disrupting the expression of the endogenous gene. The transgene may also be selectively expressed in a particular cell type with concomitant inactivation of the endogenous anaphylatoxin receptor (e.g., C3aR, C5aR) gene in only that cell type, by following, for example, the teaching of Gu *et al.* ((1994) *Science* 265:103-106). The regulatory sequences required for such a cell-type specific recombination will depend upon the particular cell type of interest and will be apparent to those of skill in the art.

[0064] Once founder animals have been generated, standard analytical techniques such as Southern blot analysis or PCR techniques are used to analyze animal tissues to determine whether integration of the transgene has taken place. The level of mRNA expression of the transgene in the tissues of the founder animals may also be assessed using techniques which include, but are not limited to, Northern blot analysis of tissue samples obtained from the animal, *in situ* hybridization analysis, and RT-PCR. Samples of anaphylatoxin receptor (e.g., C3aR, C5aR) gene-expressing tissue, may also be evaluated immunocytochemically using antibodies specific for the anaphylatoxin receptor transgene product.

[0065] An agent identified as described herein (e.g., an anaphylatoxin receptor (e.g., C3aR, C5aR) modulating agent, an antisense anaphylatoxin receptor (e.g., C3aR, C5aR) nucleic acid molecule, an anaphylatoxin receptor-specific antibody, or an anaphylatoxin receptor-binding partner) can be used in an animal model described above to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal model to determine the mechanism of action of such an agent. Furthermore, this invention pertains to uses of novel agents identified by the above-described screening assays for treatments as described herein.

#### Predictive Medicine

[0066] The invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the invention relates to diagnostic assays for detecting polypeptide and/or nucleic acid expression of markers for insulin resistance (e.g., OPN, MCP-1, HAP) as well as determining anaphylatoxin receptor (e.g., C3aR, C5aR) activity, in the context of a biological sample (e.g., blood, serum, cells, or tissue) to thereby determine whether an individual is afflicted with an insulin resistance related disease or disorder, or is at risk of developing an insulin resistance related disorder, associated with aberrant or unwanted anaphylatoxin receptor (e.g., C3aR, C5aR) expression or activity. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with insulin resistance. For example, mutations in an anaphylatoxin receptor (e.g., C3aR, C5aR) gene can be assayed in a biological sample. Additionally, assays can be used to determine serum levels of acute and chronic inflammatory markers (e.g., OPN, HAP, MCP-1) to determine susceptibility and or

progression of disease. Such assays can be used for a prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a disorder characterized by or associated with anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide activity or nucleic acid expression.

[0067] Another aspect of the invention pertains to monitoring the influence of agents (e.g., drugs, compounds) on the expression or activity of anaphylatoxin receptor (e.g., C3aR, C5aR) in clinical trials. For example, chronic inflammatory markers (e.g., OPN, HAP, MCP-1) levels can be measured as a determinant for progression of disease, and/or response to therapy. These and other agents are described in further detail in the following sections.

#### Diagnostic Assays For Metabolic Disorders

[0068] An exemplary method for detecting the presence or absence of anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide or nucleic acid in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide or nucleic acid (e.g., mRNA, or genomic DNA) that encodes anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide, such that the presence of anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide or nucleic acid is detected in the biological sample. In another aspect, the invention provides a method for detecting the presence of anaphylatoxin receptor (e.g., C3aR, C5aR) activity in a biological sample by contacting the biological sample with an agent capable of detecting an indicator of anaphylatoxin receptor (e.g., C3aR, C5aR) activity such that the presence of anaphylatoxin receptor (e.g., C3aR, C5aR) activity is detected in the biological sample.

[0069] Another exemplary method comprises detection of chronic inflammatory marker (e.g., OPN, HAP, MCP-1) levels as a determinant for progression of disease. Thus, detection of polypeptide or nucleic acid of chronic inflammatory markers (e.g., osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1)) in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting chronic inflammatory marker (e.g., osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1) polypeptide or nucleic acid (e.g., mRNA, or genomic DNA) that encodes chronic inflammatory marker (e.g., osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1) polypeptide, such that the presence of chronic inflammatory

marker (e.g., osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1) polypeptide or nucleic acid is detected in the biological sample.

[0070] A preferred agent for detecting anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammatory marker (e.g., osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1) mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammatory marker (e.g., osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1) mRNA or genomic DNA. The nucleic acid probe can be, for example, the anaphylatoxin receptor (e.g., C3aR, C5aR) nucleic acid set forth in SEQ ID NO:1 or SEQ ID NO:3; or the chronic inflammatory marker (e.g., osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1) nucleic acid set forth in SEQ ID NO:5, SEQ ID NO:7 or SEQ ID NO:17, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammatory marker (e.g., osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1) mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

[0071] A preferred agent for detecting anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammatory marker (e.g., osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1) polypeptide is an antibody capable of binding to anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide, or chronic inflammatory marker (e.g., osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1) polypeptide, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (e.g., Fab or F(ab')<sub>2</sub>) can be used. The term "label", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin.

[0072] The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject.

That is, the detection method of the invention can be used to detect anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammatory marker (e.g., osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1) mRNA, polypeptide, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammatory marker (e.g., osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1) mRNA include Northern hybridizations, *in situ* hybridizations, RT-PCR, and Taqman analyses. *In vitro* techniques for detection of anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammatory marker (e.g., osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1) polypeptide include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. *In vitro* techniques for detection of anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammatory marker (e.g., osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1) genomic DNA include Southern hybridizations.

Furthermore, *in vivo* techniques for detection of anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammatory marker (e.g., osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1) polypeptide include introducing into a subject a labeled anti-anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammatory marker (e.g., osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1) antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

**[0073]** The invention also provides diagnostic assays for identifying the presence or absence of a genetic alteration characterized by at least one of: (i) aberrant modification or mutation of a gene encoding an anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide; (ii) aberrant expression of a gene encoding an anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide; (iii) mis-regulation of the gene; or (iv) aberrant post-translational modification of an anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide, wherein a wild-type form of the gene encodes a polypeptide with an anaphylatoxin receptor (e.g., C3aR, C5aR) activity. "Misexpression or aberrant expression", as used herein, refers to a non-wild type pattern of gene expression, at the RNA or protein level. It includes, but is not limited to, expression at non-wild type levels (e.g., over or under expression); a pattern of expression that differs from wild type in terms of the time or stage at which the gene is expressed (e.g., increased or decreased expression (as compared with wild type) at a predetermined developmental period

or stage); a pattern of expression that differs from wild type in terms of decreased expression (as compared with wild type) in a predetermined cell type or tissue type; a pattern of expression that differs from wild type in terms of the splicing size, amino acid sequence, post-transitional modification, or biological activity of the expressed polypeptide; a pattern of expression that differs from wild type in terms of the effect of an environmental stimulus or extracellular stimulus on expression of the gene (*e.g.*, a pattern of increased or decreased expression (as compared with wild type) in the presence of an increase or decrease in the strength of the stimulus).

**[0074]** In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a serum sample isolated by conventional means from a subject.

**[0075]** In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting anaphylatoxin receptor (*e.g.*, C3aR, C5aR) or chronic inflammatory marker (*e.g.*, osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1) polypeptide, mRNA, or genomic DNA, such that the presence of anaphylatoxin receptor (*e.g.*, C3aR, C5aR) or chronic inflammatory marker (*e.g.*, osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1) polypeptide, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of anaphylatoxin receptor (*e.g.*, C3aR, C5aR) or chronic inflammatory marker (*e.g.*, osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1) polypeptide, mRNA or genomic DNA in the control sample with the presence of anaphylatoxin receptor (*e.g.*, C3aR, C5aR) or chronic inflammatory marker (*e.g.*, osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1) polypeptide, mRNA or genomic DNA in the test sample.

**[0076]** The invention also encompasses kits for detecting the presence of anaphylatoxin receptor (*e.g.*, C3aR, C5aR) or chronic inflammatory marker (*e.g.*, osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1) in a biological sample. For example, the kit can comprise a labeled compound or agent capable of detecting any one or more of anaphylatoxin receptor (*e.g.*, C3aR, C5aR) or chronic inflammatory marker (*e.g.*, osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1) polypeptide or mRNA in a biological sample; means for determining the amount of

anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammatory marker (e.g., osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1) in the sample; and means for comparing the amount of anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammatory marker (e.g., osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1) in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammatory marker (e.g., osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1) polypeptide or nucleic acid.

#### Prognostic Assays For Metabolic Disorders

**[0077]** The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant or unwanted anaphylatoxin receptor (e.g., C3aR, C5aR) expression or activity. As used herein, the term "aberrant" includes an anaphylatoxin receptor (e.g., C3aR, C5aR) expression or activity which deviates from the wild type anaphylatoxin receptor (e.g., C3aR, C5aR) expression or activity. Aberrant expression or activity includes increased or decreased expression or activity, as well as expression or activity which does not follow the wild type developmental pattern of expression or the subcellular pattern of expression. For example, aberrant anaphylatoxin receptor (e.g., C3aR, C5aR) expression or activity is intended to include the cases in which a mutation in the anaphylatoxin receptor (e.g., C3aR, C5aR) gene causes the anaphylatoxin receptor (e.g., C3aR, C5aR) gene to be under-expressed or over-expressed and situations in which such mutations result in a non-functional anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide or a polypeptide which does not function in a wild-type fashion, e.g., a polypeptide which does not interact with an anaphylatoxin receptor (e.g., C3aR, C5aR) ligand, e.g., anaphylatoxin (e.g., C3a, C5a), or one which interacts with a non-anaphylatoxin receptor (e.g., C3aR, C5aR) ligand. As used herein, the term "unwanted" includes an unwanted phenomenon involved in a biological response, such as cellular proliferation. For example, the term "unwanted" includes an anaphylatoxin receptor (e.g., C3aR, C5aR) expression or activity which is undesirable in a subject.

**[0078]** The assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having, or at risk of developing, a disorder associated with a misregulation in anaphylatoxin receptor (e.g., C3aR, C5aR)

polypeptide activity or nucleic acid expression, such as a metabolic disorder. Alternatively, the prognostic assays can be utilized to identify a subject having, or at risk for developing, a disorder associated with a misregulation in anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide activity or nucleic acid expression, such as a metabolic disorder. Thus, the invention provides a method for identifying a disease or disorder associated with aberrant or unwanted anaphylatoxin receptor (e.g., C3aR, C5aR) expression or activity in which a test sample is obtained from a subject and anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide or nucleic acid (*e.g.*, mRNA or genomic DNA) is detected, wherein the presence of anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide or nucleic acid is diagnostic for a subject having, or at risk of developing, a disease or disorder associated with aberrant or unwanted anaphylatoxin receptor (e.g., C3aR, C5aR) expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (*e.g.*, serum), cell sample, or tissue.

**[0079]** Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (*e.g.*, an activator, inhibitor, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant or unwanted anaphylatoxin receptor (e.g., C3aR, C5aR) expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for a metabolic disorder. Thus, the invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant or unwanted anaphylatoxin receptor (e.g., C3aR, C5aR) expression or activity in which a test sample is obtained and anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide or nucleic acid expression or activity is detected (*e.g.*, wherein the abundance of anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide or nucleic acid expression or activity is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant or unwanted anaphylatoxin receptor (e.g., C3aR, C5aR) expression or activity).

**[0080]** The methods of the invention can also be used to detect genetic alterations in an anaphylatoxin receptor (e.g., C3aR, C5aR) gene, thereby determining if a subject with the altered gene is at risk for a disorder characterized by misregulation in anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide activity or nucleic acid expression, such as a metabolic disorder. In preferred embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic alteration characterized by at least one

of an alteration affecting the integrity of a gene encoding a anaphylatoxin receptor - polypeptide, or the mis-expression of the anaphylatoxin receptor (e.g., C3aR, C5aR) gene. For example, such genetic alterations can be detected by ascertaining the existence of at least one of: (1) a deletion of one or more nucleotides from a anaphylatoxin receptor (e.g., C3aR, C5aR) gene; (2) an addition of one or more nucleotides to a anaphylatoxin receptor (e.g., C3aR, C5aR) gene; (3) a substitution of one or more nucleotides of an anaphylatoxin receptor (e.g., C3aR, C5aR) gene; (4) a chromosomal rearrangement of an anaphylatoxin receptor (e.g., C3aR, C5aR) gene; (5) an alteration in the level of a messenger RNA transcript of an anaphylatoxin receptor (e.g., C3aR, C5aR) gene; (6) aberrant modification of an anaphylatoxin receptor (e.g., C3aR, C5aR) gene, such as of the methylation pattern of the genomic DNA; (7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of a anaphylatoxin receptor (e.g., C3aR, C5aR) gene; (8) a non-wild type level of an anaphylatoxin receptor-polypeptide; (9) allelic loss of an anaphylatoxin receptor (e.g., C3aR, C5aR) gene; and (10) inappropriate post-translational modification of an anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide. As described herein, there are a large number of assays known in the art which can be used for detecting alterations in an anaphylatoxin receptor (e.g., C3aR, C5aR) gene. A preferred biological sample is a tissue or serum sample isolated, e.g., by conventional means, from a subject.

**[0081]** In certain embodiments, detection of the alteration involves the use of a probe/primer in a polymerase chain reaction (PCR) (see, e.g., U.S. Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (see, e.g., Landegran *et al.* (1988) *Science* 241:1077-1080; and Nakazawa *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:360-364), the latter of which can be particularly useful for detecting point mutations in the anaphylatoxin receptor (e.g., C3aR, C5aR) gene (see Abravaya *et al.* (1995) *Nucleic Acids Res.* 23:675-682). This method can include the steps of collecting a sample of cells from a subject, isolating nucleic acid (e.g., genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to a anaphylatoxin receptor (e.g., C3aR, C5aR) gene under conditions such that hybridization and amplification of the anaphylatoxin receptor (e.g., C3aR, C5aR) gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. PCR and/or LCR sensitivity can be enhanced by use of a

preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

**[0082]** Alternative amplification methods include: self sustained sequence replication (Guatelli, J.C. *et al.*, (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh, D. Y. *et al.*, (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi, P.M. *et al.* (1988) *Bio-Technology* 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

**[0083]** In an alternative embodiment, mutations in an anaphylatoxin receptor (e.g., C3aR, C5aR) gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA are isolated, optionally amplified, then digested with one or more restriction endonucleases. Fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicate mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see, for example, U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

**[0084]** In other embodiments, genetic mutations in anaphylatoxin receptor (e.g., C3aR, C5aR) can be identified by hybridizing a sample and control nucleic acids, *e.g.*, DNA or RNA, to high density arrays containing hundreds or thousands of oligonucleotides probes (Cronin, M.T. *et al.* (1996) *Human Mutation* 7: 244-255; Kozal, M. J. *et al.* (1996) *Nature Medicine* 2: 753-759). For example, genetic mutations in anaphylatoxin receptor (e.g., C3aR, C5aR) can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin, M.T. *et al. supra*. Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

**[0085]** In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the anaphylatoxin receptor (e.g., C3aR, C5aR) gene and detect mutations by comparing the sequence of the sample anaphylatoxin receptor (e.g., C3aR, C5aR) with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxam and Gilbert ((1977) *Proc. Natl. Acad. Sci. USA* 74:560) or Sanger ((1977) *Proc. Natl. Acad. Sci. USA* 74:5463). It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays ((1995) *Biotechniques* 19:448), including sequencing by mass spectrometry (see, e.g., PCT Publication No. WO 94/16101; Cohen *et al.* (1996) *Adv. Chromatogr.* 36:127-162; and Griffin *et al.* (1993) *Appl. Biochem. Biotechnol.* 38:147-159).

**[0086]** Other methods for detecting mutations in the anaphylatoxin receptor (e.g., C3aR, C5aR) gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers *et al.* (1985) *Science* 230:1242). In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes formed by hybridizing (labeled) RNA or DNA containing the wild-type anaphylatoxin receptor (e.g., C3aR, C5aR) sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent which cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S1 nuclease to enzymatically digest the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. See, for example, Cotton *et al.* (1988) *Proc. Natl Acad Sci USA* 85:4397; Saleeba *et al.* (1992) *Methods Enzymol.* 217:286-295. In a preferred embodiment, the control DNA or RNA can be labeled for detection.

**[0087]** In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in anaphylatoxin receptor (e.g., C3aR, C5aR) cDNAs obtained from samples of cells. For example, the mutY enzyme of E. coli cleaves A at G/A mismatches and the

thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu *et al.* (1994) *Carcinogenesis* 15:1657-1662). According to an exemplary embodiment, a probe based on a anaphylatoxin receptor (e.g., C3aR, C5aR) sequence, *e.g.*, a wild-type anaphylatoxin receptor (e.g., C3aR, C5aR) sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. See, for example, U.S. Patent No. 5,459,039.

**[0088]** In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in anaphylatoxin receptor (e.g., C3aR, C5aR) genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita *et al.* (1989) *Proc Natl. Acad. Sci USA*: 86:2766, see also Cotton (1993) *Mutat. Res.* 285:125-144; and Hayashi (1992) *Genet. Anal. Tech. Appl.* 9:73-79). Single-stranded DNA fragments of sample and control anaphylatoxin receptor (e.g., C3aR, C5aR) nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In a preferred embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen *et al.* (1991) *Trends Genet* 7:5).

**[0089]** In yet another embodiment the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers *et al.* (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example, by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) *Biophys Chem* 265:12753).

**[0090]** Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the

known mutation is placed centrally and then hybridized to target DNA under conditions which permit hybridization only if a perfect match is found (Saiki *et al.* (1986) *Nature* 324:163); Saiki *et al.* (1989) *Proc. Natl Acad. Sci USA* 86:6230). Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

[0091] Alternatively, allele specific amplification technology which depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs *et al.* (1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition, it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini *et al.* (1992) *Mol. Cell Probes* 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) *Proc. Natl. Acad. Sci USA* 88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

[0092] The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, *e.g.*, in clinical settings to diagnose patients exhibiting symptoms or family history of a metabolic disease or illness involving an anaphylatoxin receptor (*e.g.*, C3aR, C5aR) gene.

[0093] Furthermore, any cell type or tissue in which anaphylatoxin receptor (*e.g.*, C3aR, C5aR) is expressed may be utilized in the prognostic assays described herein.

#### Monitoring of Effects During Clinical Trials

[0094] Monitoring the influence of agents (*e.g.*, drugs) on the expression or activity of an anaphylatoxin receptor (*e.g.*, C3aR, C5aR) polypeptide (*e.g.*, the modulation of an enzymatic or catalytic activity) can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent determined by a screening assay as

described herein to increase anaphylatoxin receptor (e.g., C3aR, C5aR) gene expression, polypeptide levels, or upregulate anaphylatoxin receptor (e.g., C3aR, C5aR) activity, can be monitored in clinical trials of subjects exhibiting decreased anaphylatoxin receptor (e.g., C3aR, C5aR) gene expression, polypeptide levels, or downregulated anaphylatoxin receptor (e.g., C3aR, C5aR) activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease anaphylatoxin receptor (e.g., C3aR, C5aR) gene expression, polypeptide levels, or downregulate anaphylatoxin receptor (e.g., C3aR, C5aR) activity, can be monitored in clinical trials of subjects exhibiting increased anaphylatoxin receptor (e.g., C3aR, C5aR) gene expression, polypeptide levels, or upregulated anaphylatoxin receptor (e.g., C3aR, C5aR) activity. In such clinical trials, the expression or activity of an anaphylatoxin receptor (e.g., C3aR, C5aR) gene, and preferably, other genes that have been implicated in, for example, an anaphylatoxin receptor -associated disorder, e.g., a metabolic disease or disorder, can be used as a "read out" or markers of the phenotype of a particular cell. In certain embodiments, detection of chronic inflammatory marker (e.g., OPN, HAP, MCP-1) levels can be used a determinant or "read out" indicator of activity of anaphylatoxin receptor (e.g., C3aR, C5aR) activity, and/or progression of disease. Thus, detection of polypeptide or nucleic acid of chronic inflammatory markers (e.g., osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1)) in a biological sample can be used as an indicator of the influence of agents and monitoring during clinical trials.

**[0095]** For example, and not by way of limitation, genes, including anaphylatoxin receptor, that are modulated in cells by treatment with an agent (e.g., compound, drug or small molecule) which modulates anaphylatoxin receptor (e.g., C3aR, C5aR) activity (e.g., identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents on metabolic disorders, for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of anaphylatoxin receptor (e.g., C3aR, C5aR) and other genes implicated in the metabolic disorder, respectively. The levels of gene expression (e.g., a gene expression pattern) can be quantified by northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of polypeptide produced, by one of the methods as described herein, or by measuring the levels of activity of anaphylatoxin receptor (e.g., C3aR, C5aR) or other genes. In this way, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during treatment of the individual with the agent.

**[0096]** In a preferred embodiment, the invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (*e.g.*, an activator (*e.g.*, agonist), inhibitor (*e.g.*, antagonist), peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) including the steps of: (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of expression of an anaphylatoxin receptor (*e.g.*, C3aR, C5aR) polypeptide, mRNA, or genomic DNA in the preadministration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level of expression or activity of the anaphylatoxin receptor (*e.g.*, C3aR, C5aR) polypeptide, mRNA, or genomic DNA in the post-administration samples; (v) comparing the level of expression or activity of the anaphylatoxin receptor (*e.g.*, C3aR, C5aR) polypeptide, mRNA, or genomic DNA in the pre-administration sample with the anaphylatoxin receptor (*e.g.*, C3aR, C5aR) polypeptide, mRNA, or genomic DNA in the post administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of anaphylatoxin receptor (*e.g.*, C3aR, C5aR) to higher levels than detected, *i.e.*, to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of anaphylatoxin receptor (*e.g.*, C3aR, C5aR) to lower levels than detected, *i.e.* to decrease the effectiveness of the agent. According to such an embodiment, anaphylatoxin receptor (*e.g.*, C3aR, C5aR) expression or activity may be used as an indicator of the effectiveness of an agent, even in the absence of an observable phenotypic response.

**[0097]** Similarly, detection of one or more chronic inflammatory marker (*e.g.*, OPN, HAP, MCP-1) levels can be carried out as described using similar methods as those described above, adapted for the particular marker of interest.

#### Electronic Apparatus Readable Media and Arrays

**[0098]** Electronic apparatus readable media comprising anaphylatoxin receptor (*e.g.*, C3aR, C5aR) sequence information is also provided. As used herein, “sequence information” refers to any nucleotide and/or amino acid sequence information particular to the anaphylatoxin receptor (*e.g.*, C3aR, C5aR) or chronic inflammatory marker (*e.g.*, osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1) molecules of the invention, including but not limited to full-length nucleotide and/or amino

acid sequences, partial nucleotide and/or amino acid sequences, polymorphic sequences including single nucleotide polymorphisms (SNPs), epitope sequences, and the like. Moreover, information “related to” said anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammatory marker (e.g., osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1) sequence information includes detection of the presence or absence of a sequence (e.g., detection of expression of a sequence, fragment, polymorphism, etc.), determination of the level of a sequence (e.g., detection of a level of expression, for example, a quantitative detection), detection of a reactivity to a sequence (e.g., detection of protein expression and/or levels, for example, using a sequence-specific antibody), and the like. As used herein, “electronic apparatus readable media” refers to any suitable medium for storing, holding or containing data or information that can be read and accessed directly by an electronic apparatus. Such media can include, but are not limited to: magnetic storage media, such as floppy discs, hard disc storage medium, and magnetic tape; optical storage media such as compact disc; electronic storage media such as RAM, ROM, EPROM, EEPROM and the like; general hard disks and hybrids of these categories such as magnetic/optical storage media. The medium is adapted or configured for having recorded thereon anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammatory marker (e.g., osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1) sequence information of the invention.

**[0099]** As used herein, the term “electronic apparatus” is intended to include any suitable computing or processing apparatus or other device configured or adapted for storing data or information. Examples of electronic apparatus suitable for use with the invention include stand-alone computing apparatus; networks, including a local area network (LAN), a wide area network (WAN) Internet, Intranet, and Extranet; electronic appliances such as a personal digital assistants (PDAs), cellular phone, pager and the like; and local and distributed processing systems.

**[00100]** As used herein, “recorded” refers to a process for storing or encoding information on the electronic apparatus readable medium. Those skilled in the art can readily adopt any of the presently known methods for recording information on known media to generate manufactures comprising the anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammatory marker (e.g., osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1)) sequence information.

[00101] A variety of software programs and formats can be used to store the sequence information on the electronic apparatus readable medium. For example, the sequence information can be represented in a word processing text file, formatted in commercially-available software such as WordPerfect and MicroSoft Word, or represented in the form of an ASCII file, stored in a database application, such as DB2, Sybase, Oracle, or the like, as well as in other forms. Any number of dataprocessor structuring formats (*e.g.*, text file or database) may be employed in order to obtain or create a medium having recorded thereon the anaphylatoxin receptor (*e.g.*, C3aR, C5aR) or chronic inflammatory marker (*e.g.*, osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1)) sequence information.

[00102] By providing anaphylatoxin receptor (*e.g.*, C3aR, C5aR) or chronic inflammatory marker (*e.g.*, osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1)) sequence information in readable form, one can routinely access the sequence information for a variety of purposes. For example, one skilled in the art can use the sequence information in readable form to compare a target sequence or target structural motif with the sequence information stored within the data storage means. Search means are used to identify fragments or regions of the sequences of the invention which match a particular target sequence or target motif.

[00103] The invention therefore provides a medium for holding instructions for performing a method for determining whether a subject has an anaphylatoxin receptor - associated, *e.g.*, a metabolic, disease or disorder or a pre-disposition to an anaphylatoxin receptor -associated, *e.g.*, a metabolic, disease or disorder, wherein the method comprises the steps of determining anaphylatoxin receptor (*e.g.*, C3aR, C5aR) or chronic inflammatory marker (*e.g.*, osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1)) sequence information associated with the subject and based on the anaphylatoxin receptor (*e.g.*, C3aR, C5aR) or chronic inflammatory marker (*e.g.*, osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1)) sequence information, determining whether the subject has an anaphylatoxin receptor -associated disease or disorder or a pre-disposition to an anaphylatoxin receptor -associated disease or disorder and/or recommending a particular treatment for the disease, disorder or pre-disease condition.

[00104] The invention further provides in an electronic system and/or in a network, a method for determining whether a subject has an anaphylatoxin receptor -associated, *e.g.*, a

metabolic, disease or disorder or a pre-disposition to a disease associated with an anaphylatoxin receptor, wherein the method comprises the steps of determining anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammatory marker (e.g., osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1)) sequence information associated with the subject, and based on the anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammatory marker (e.g., osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1)) sequence information, determining whether the subject has an anaphylatoxin receptor -associated disease or disorder or a pre-disposition to an anaphylatoxin receptor -associated disease or disorder, and/or recommending a particular treatment for the disease, disorder or pre-disease condition. The method may further comprise the step of receiving phenotypic information associated with the subject and/or acquiring from a network phenotypic information associated with the subject.

**[00105]** The invention also provides in a network, a method for determining whether a subject has an anaphylatoxin receptor -associated, e.g., a metabolic, disease or disorder or a pre-disposition to an anaphylatoxin receptor -associated disease or disorder associated with anaphylatoxin receptor, said method comprising the steps of receiving anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammatory marker (e.g., osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1)) sequence information from the subject and/or information related thereto, receiving phenotypic information associated with the subject, acquiring information from the network corresponding to anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammatory marker (e.g., osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1)) and/or an anaphylatoxin receptor -associated disease or disorder, and based on one or more of the phenotypic information, the anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammatory marker (e.g., osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1)) information (e.g., sequence information and/or information related thereto), and the acquired information, determining whether the subject has an anaphylatoxin receptor-associated disease or disorder or a pre-disposition to an anaphylatoxin receptor-associated disease or disorder. The method may further comprise the step of recommending a particular treatment for the disease, disorder or pre-disease condition.

[00106] The invention also provides a business method for determining whether a subject has an anaphylatoxin receptor-associated, *e.g.*, a metabolic, disease or disorder or a predisposition to an anaphylatoxin receptor-associated disease or disorder, said method comprising the steps of receiving information related to anaphylatoxin receptor (*e.g.*, C3aR, C5aR) (*e.g.*, sequence information and/or information related thereto), receiving phenotypic information associated with the subject, acquiring information from the network related to anaphylatoxin receptor (*e.g.*, C3aR, C5aR) or chronic inflammatory marker (*e.g.*, osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1)) and/or related to an anaphylatoxin receptor-associated disease or disorder, and based on one or more of the phenotypic information, the anaphylatoxin receptor (*e.g.*, C3aR, C5aR) or chronic inflammatory marker (*e.g.*, osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1)) information, and the acquired information, determining whether the subject has an anaphylatoxin receptor-associated disease or disorder or a predisposition to an anaphylatoxin receptor-associated disease or disorder. The method may further comprise the step of recommending a particular treatment for the disease, disorder or pre-disease condition.

[00107] The invention also includes an array comprising an anaphylatoxin receptor (*e.g.*, C3aR, C5aR) or chronic inflammatory marker (*e.g.*, osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1)) sequence of the invention. The array can be used to assay expression of one or more genes in the array. In one embodiment, the array can be used to assay gene expression in a tissue to ascertain tissue specificity of genes in the array. In this manner, up to about 7600 genes can be simultaneously assayed for expression, one of which can be anaphylatoxin receptor. This allows a profile to be developed showing a battery of genes specifically expressed in one or more tissues.

[00108] In addition to such qualitative determination, the invention allows the quantitation of gene expression. Thus, not only tissue specificity, but also the level of expression of a battery of genes in the tissue is ascertainable. Thus, genes can be grouped on the basis of their tissue expression *per se* and level of expression in that tissue. This is useful, for example, in ascertaining the relationship of gene expression between or among tissues. Thus, one tissue can be perturbed and the effect on gene expression in a second tissue can be determined. In this context, the effect of one cell type on another cell type in response to a biological stimulus can be determined. Such a determination is useful, for example, for determining the effect of cell-cell interactions at the level of gene expression.

If an agent is administered therapeutically to treat one cell type but has an undesirable effect on another cell type, the invention provides an assay to determine the molecular basis of the undesirable effect and thus provides the opportunity to co-administer a counteracting agent or otherwise treat the undesired effect. Similarly, even within a single cell type, undesirable biological effects can be determined at the molecular level. Thus, the effects of an agent on expression of other than the target gene can be ascertained and counteracted.

**[00109]** In another embodiment, the array can be used to monitor the time course of expression of one or more genes in the array. This can occur in various biological contexts, as disclosed herein, for example development of an anaphylatoxin receptor-associated, *e.g.*, a metabolic, disease or disorder, progression of anaphylatoxin receptor-associated disease or disorder, and processes, such a cellular transformation associated with the anaphylatoxin receptor-associated disease or disorder.

**[00110]** The array is also useful for ascertaining the effect of the expression of a gene on the expression of other genes in the same cell or in different cells (*e.g.*, ascertaining the effect of anaphylatoxin receptor (*e.g.*, C3aR, C5aR) or chronic inflammatory marker (*e.g.*, osteopontin (OPN), haptoglobin (HAP), or macrophage chemotactic protein-1 (MCP-1)) expression on the expression of other genes). This provides, for example, for a selection of alternate molecular targets for therapeutic intervention if the ultimate or downstream target cannot be regulated.

**[00111]** The array is also useful for ascertaining differential expression patterns of one or more genes in normal and abnormal cells. This provides a battery of genes (*e.g.*, including anaphylatoxin receptor) that could serve as a molecular target for diagnosis or therapeutic intervention.

#### Methods of Treatment of Subjects Suffering From Metabolic Disorders

**[00112]** The invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant or unwanted anaphylatoxin receptor (*e.g.*, C3aR, C5aR) expression or activity, *e.g.* a metabolic disorder such as obesity or diabetes. With regard to both prophylactic and therapeutic methods of treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics. "Pharmacogenomics", as used herein, refers to the application of genomics technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the

market. More specifically, the term refers the study of how a patient's genes determine his or her response to a drug (*e.g.*, a patient's "drug response phenotype", or "drug response genotype"). Thus, another aspect of the invention provides methods for tailoring an individual's prophylactic or therapeutic treatment with either the anaphylatoxin receptor (*e.g.*, C3aR, C5aR) molecules of the invention or anaphylatoxin receptor (*e.g.*, C3aR, C5aR) modulators according to that individual's drug response genotype. Pharmacogenomics allows a clinician or physician to target prophylactic or therapeutic treatments to patients who will most benefit from the treatment and to avoid treatment of patients who will experience toxic drug-related side effects.

**[00113]** Treatment is defined as the application or administration of a therapeutic agent to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient, who has a disease, a symptom of disease or a predisposition toward a disease, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disease, the symptoms of disease or the predisposition toward disease.

**[00114]** A therapeutic agent includes, but is not limited to, small molecules, peptides, antibodies, ribozymes and antisense oligonucleotides.

#### Prophylactic Methods

**[00115]** In one aspect, the invention provides a method for preventing in a subject, a disease or condition associated with an aberrant or unwanted anaphylatoxin receptor (*e.g.*, C3aR, C5aR) expression or activity, by administering to the subject an anaphylatoxin receptor (*e.g.*, C3aR, C5aR) or an agent which modulates anaphylatoxin receptor (*e.g.*, C3aR, C5aR) expression or at least one anaphylatoxin receptor (*e.g.*, C3aR, C5aR) activity. Subjects at risk for a disease which is caused or contributed to by aberrant or unwanted anaphylatoxin receptor (*e.g.*, C3aR, C5aR) expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the anaphylatoxin receptor (*e.g.*, C3aR, C5aR) aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of anaphylatoxin receptor (*e.g.*, C3aR, C5aR) aberrancy, for example, an anaphylatoxin receptor (*e.g.*, C3aR, C5aR) molecule, anaphylatoxin receptor (*e.g.*, C3aR, C5aR) agonist or anaphylatoxin receptor (*e.g.*, C3aR, C5aR) antagonist agent can be used

for treating the subject. The appropriate agent can be determined based on screening assays described herein.

### Therapeutic Methods

**[00116]** The anaphylatoxin receptor (e.g., C3aR, C5aR) nucleic acid molecules, fragments of anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptides, and anti-anaphylatoxin receptor (e.g., C3aR, C5aR) antibodies (also referred to herein as "active compounds") of the invention can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, polypeptide, or antibody and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

**[00117]** A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal or topical, transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

**[00118]** Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration,

suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

**[00119]** Sterile injectable solutions can be prepared by incorporating the active compound (e.g., a fragment of an anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide or an anti-anaphylatoxin receptor (e.g., C3aR, C5aR) antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

**[00120]** Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The

tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

[00121] For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

[00122] For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

[00123] The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

[00124] In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially, for example, from Alza Corporation (Palo Alto, CA) or Alkermes (Cambridge, MA). Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

[00125] It is especially advantageous to formulate oral or parenteral compositions in “dosage unit form” for ease of administration and uniformity of dosage. “Dosage unit form”, as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound

calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

[00126] Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD<sub>50</sub> (the dose lethal to 50% of the population) and the ED<sub>50</sub> (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD<sub>50</sub>/ED<sub>50</sub>. Compounds which exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

[00127] The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED<sub>50</sub> with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC<sub>50</sub> (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

[00128] As defined herein, a therapeutically effective amount of polypeptide (*i.e.*, an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight. The skilled artisan will appreciate that certain factors may influence the dosage required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective

amount of a polypeptide or antibody can include a single treatment or, preferably, can include a series of treatments.

[00129] In a preferred example, a subject is treated with antibody or polypeptide in the range of between about 0.1 to 20 mg/kg body weight, one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. It will also be appreciated that the effective dosage of antibody or polypeptide used for treatment may increase or decrease over the course of a particular treatment. Changes in dosage may result and become apparent from the results of diagnostic assays as described herein.

[00130] The invention encompasses agents which modulate expression or activity. An agent may, for example, be a small molecule. For example, such small molecules include, but are not limited to, peptides, peptidomimetics, amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (*i.e.*, including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds. It is understood that appropriate doses of small molecule agents depends upon a number of factors within the ken of the ordinarily skilled physician, veterinarian, or researcher. The dose(s) of the small molecule will vary, for example, depending upon the identity, size, and condition of the subject or sample being treated, further depending upon the route by which the composition is to be administered, if applicable, and the effect which the practitioner desires the small molecule to have upon the nucleic acid or polypeptide of the invention.

[00131] Exemplary doses include milligram or microgram amounts of the small molecule per kilogram of subject or sample weight (*e.g.*, about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram. It is furthermore understood that appropriate doses of a small molecule depend upon the potency of the small molecule with respect to the expression or activity to be modulated. Such appropriate doses may be determined using the assays described herein. When one or more of these small molecules is to be administered to an animal (*e.g.*, a human) in order to

modulate expression or activity of a polypeptide or nucleic acid of the invention, a physician, veterinarian, or researcher may, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. In addition, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity to be modulated.

[00132] Further, an antibody (or fragment thereof) may be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent or a radioactive metal ion. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicine, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologues thereof. Therapeutic agents include, but are not limited to, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine and vinblastine).

[00133] The conjugates of the invention can be used for modifying a given biological response, the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, alpha-interferon, beta-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

[00134] Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson *et al.* (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin *et al.* (eds.), pp. 303-16 (Academic Press 1985), and Thorpe *et al.*, "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev., 62:119-58 (1982). Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980.

[00135] The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by stereotactic injection (see e.g., Chen *et al.* (1994) Proc. Natl. Acad. Sci. USA 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g., retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

[00136] The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

#### Pharmacogenomics

[00137] The anaphylatoxin receptor (e.g., C3aR, C5aR) molecules of the invention, as well as agents, or modulators which have a stimulatory or inhibitory effect on anaphylatoxin receptor (e.g., C3aR, C5aR) activity (e.g., anaphylatoxin receptor (e.g., C3aR, C5aR) gene expression) as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) metabolic disorders (e.g., proliferative disorders) associated with aberrant or unwanted anaphylatoxin receptor (e.g.,

C3aR, C5aR) activity. In conjunction with such treatment, pharmacogenomics (*i.e.*, the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, a physician or clinician may consider applying knowledge obtained in relevant pharmacogenomics studies in determining whether to administer a anaphylatoxin receptor (*e.g.*, C3aR, C5aR) molecule or anaphylatoxin receptor (*e.g.*, C3aR, C5aR) modulator as well as tailoring the dosage and/or therapeutic regimen of treatment with a anaphylatoxin receptor (*e.g.*, C3aR, C5aR) molecule or anaphylatoxin receptor (*e.g.*, C3aR, C5aR) modulator.

**[00138]** Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See, for example, Eichelbaum, M. *et al.* (1996) *Clin. Exp. Pharmacol. Physiol.* 23(10-11): 983-985 and Linder, M. W. *et al.* (1997) *Clin. Chem.* 43(2):254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare genetic defects or as naturally-occurring polymorphisms. For example, glucose-6-phosphate dehydrogenase deficiency (G6PD) is a common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

**[00139]** One pharmacogenomics approach to identifying genes that predict drug response, known as "a genome-wide association", relies primarily on a high-resolution map of the human genome consisting of already known gene-related markers (*e.g.*, a "bi-allelic" gene marker map which consists of 60,000-100,000 polymorphic or variable sites on the human genome, each of which has two variants.) Such a high-resolution genetic map can be compared to a map of the genome of each of a statistically significant number of patients taking part in a Phase II/III drug trial to identify markers associated with a particular observed drug response or side effect. Alternatively, such a high resolution map can be generated from a combination of some ten-million known single nucleotide polymorphisms (SNPs) in the human genome. As used herein, a "SNP" is a common alteration that occurs in a single nucleotide base in a stretch of DNA. For example, a SNP may occur once per

every 1000 bases of DNA. A SNP may be involved in a disease process, however, the vast majority may not be disease-associated. Given a genetic map based on the occurrence of such SNPs, individuals can be grouped into genetic categories depending on a particular pattern of SNPs in their individual genome. In such a manner, treatment regimens can be tailored to groups of genetically similar individuals, taking into account traits that may be common among such genetically similar individuals.

[00140] Alternatively, a method termed the "candidate gene approach", can be utilized to identify genes that predict drug response. According to this method, if a gene that encodes a drugs target is known (*e.g.*, an anaphylatoxin receptor (*e.g.*, C3aR, C5aR) polypeptide of the invention), all common variants of that gene can be fairly easily identified in the population and it can be determined if having one version of the gene versus another is associated with a particular drug response.

[00141] As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (*e.g.*, N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. The other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

[00142] Alternatively, a method termed the "gene expression profiling", can be utilized to identify genes that predict drug response. For example, the gene expression of an animal dosed with a drug (*e.g.*, an anaphylatoxin receptor (*e.g.*, C3aR, C5aR) molecule or

anaphylatoxin receptor (e.g., C3aR, C5aR) modulator of the invention) can give an indication whether gene pathways related to toxicity have been turned on.

**[00143]** Information generated from more than one of the above pharmacogenomics approaches can be used to determine appropriate dosage and treatment regimens for prophylactic or therapeutic treatment an individual. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with an anaphylatoxin receptor (e.g., C3aR, C5aR) molecule or anaphylatoxin receptor (e.g., C3aR, C5aR) modulator, such as a modulator identified by one of the exemplary screening assays described herein.

#### Recombinant Expression Vectors and Host Cells Used in the Methods of the Invention

**[00144]** The methods of the invention (e.g., the screening assays described herein) include the use of vectors, preferably expression vectors, containing a nucleic acid encoding an anaphylatoxin receptor (e.g., C3aR, C5aR) protein, or a portion thereof. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

**[00145]** The recombinant expression vectors to be used in the methods of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more

regulatory sequences, selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel (1990) *Methods Enzymol.* 185:3-7. Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cells and those which direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, and the like. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (*e.g.*, anaphylatoxin receptor (*e.g.*, C3aR, C5aR) proteins, mutant forms of anaphylatoxin receptor (*e.g.*, C3aR, C5aR) proteins, fusion proteins, and the like).

[00146] The recombinant expression vectors to be used in the methods of the invention can be designed for expression of anaphylatoxin receptor (*e.g.*, C3aR, C5aR) proteins in prokaryotic or eukaryotic cells. For example, anaphylatoxin receptor (*e.g.*, C3aR, C5aR) proteins can be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors), yeast cells, or mammalian cells. Suitable host cells are discussed further in Goeddel (1990) *supra*. Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

[00147] Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion

expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S. (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

**[00148]** Purified fusion proteins can be utilized in anaphylatoxin receptor (e.g., C3aR, C5aR) activity assays, (e.g., direct assays or competitive assays described in detail below), or to generate antibodies specific for anaphylatoxin receptor (e.g., C3aR, C5aR) proteins. In a preferred embodiment, an anaphylatoxin receptor (e.g., C3aR, C5aR) fusion protein expressed in a retroviral expression vector of the invention can be utilized to infect bone marrow cells which are subsequently transplanted into irradiated recipients. The pathology of the subject recipient is then examined after sufficient time has passed (e.g., six weeks).

**[00149]** In another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, B. (1987) *Nature* 329:840) and pMT2PC (Kaufman *et al.* (1987) *EMBO J.* 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook, J. *et al.*, *Molecular Cloning: A Laboratory Manual. 2nd ed.*, Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

**[00150]** In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid).

**[00151]** The methods of the invention may further use a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner which allows for expression (by transcription of the DNA molecule) of an RNA molecule which is antisense to anaphylatoxin receptor (e.g., C3aR, C5aR)

mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen which direct constitutive, tissue specific, or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid, or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes, see Weintraub, H. *et al.*, Antisense RNA as a molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1(1) 1986.

**[00152]** Another aspect of the invention pertains to the use of host cells into which an anaphylatoxin receptor (e.g., C3aR, C5aR) nucleic acid molecule of the invention is introduced, e.g., an anaphylatoxin receptor (e.g., C3aR, C5aR) nucleic acid molecule within a recombinant expression vector or an anaphylatoxin receptor (e.g., C3aR, C5aR) nucleic acid molecule containing sequences which allow it to homologously recombine into a specific site of the host cell's genome. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

**[00153]** A host cell can be any prokaryotic or eukaryotic cell. For example, a anaphylatoxin receptor (e.g., C3aR, C5aR) protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

**[00154]** Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (e.g., DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook *et al.* (*Molecular Cloning: A Laboratory*

*Manual. 2nd, ed., Cold Spring Harbor Laboratory*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989), and other laboratory manuals.

**[00155]** A host cell used in the methods of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) an anaphylatoxin receptor (e.g., C3aR, C5aR) protein. Accordingly, the invention further provides methods for producing an anaphylatoxin receptor (e.g., C3aR, C5aR) protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of the invention (into which a recombinant expression vector encoding a anaphylatoxin receptor (e.g., C3aR, C5aR) protein has been introduced) in a suitable medium such that an anaphylatoxin receptor (e.g., C3aR, C5aR) protein is produced. In another embodiment, the method further comprises isolating a anaphylatoxin receptor (e.g., C3aR, C5aR) protein from the medium or the host cell.

#### Isolated Nucleic Acid Molecules Used in the Methods of the Invention

**[00156]** The nucleotide sequence of human anaphylatoxin receptors C3aR, C5aR (GenBank Accession Nos. Z73157 and X57250) are depicted in SEQ ID NO:1 and SEQ ID NO:3, respectively. The amino acid sequence corresponds to SEQ ID NO:2 and SEQ ID NO:4.

**[00157]** The nucleotide sequence of murine anaphylatoxin receptors C3aR, and C5aR (GenBank Accession Nos. U77461 and S46665 L05630) are depicted in SEQ ID NO:9 and SEQ ID NO:11, respectively. The amino acid sequence corresponds to SEQ ID NO:10 and SEQ ID NO:12.

**[00158]** The nucleotide sequence of human haptoglobin (HAP) (GenBank Accession No. NM005143) is depicted in SEQ ID NO:5. The amino acid sequence corresponds to SEQ ID NO:6.

**[00159]** The nucleotide sequence of murine haptoglobin (HAP) (GenBank Accession No. NM017370) is depicted in SEQ ID NO:13. The amino acid sequence corresponds to SEQ ID NO:14.

**[00160]** The nucleotide sequence of human osteopontin (OPN) (GenBank Accession No. J04765) is depicted in SEQ ID NO:7. The amino acid sequence corresponds to SEQ ID NO:8.

[00161] The nucleotide sequence of murine osteopontin (OPN) (GenBank Accession No. AF515708) is depicted in SEQ ID NO:15. The amino acid sequence corresponds to SEQ ID NO:16.

[00162] The nucleotide sequence of human monocyte chemoattractant protein 1 (MCP-1) (GenBank Accession No. X14768) are depicted in SEQ ID NO:17. The amino acid sequence corresponds to SEQ ID NO:18.

[00163] The nucleotide sequence of murine monocyte chemoattractant protein 1 (MCP-1) (GenBank Accession No. L13763) are depicted in SEQ ID NO:19. The amino acid sequence corresponds to SEQ ID NO:20.

[00164] The methods of the invention include the use of isolated nucleic acid molecules that encode anaphylatoxin receptor C3aR and C5aR proteins, chronic inflammatory marker osteopontin (OPN), haptoglobin (HAP), and monocyte chemoattractant protein 1 (MCP-1) proteins, or biologically active portions thereof, as well as nucleic acid fragments sufficient for use as hybridization probes to identify anaphylatoxin receptor C3aR and C5aR encoding nucleic acid molecules or chronic inflammatory marker osteopontin (OPN), haptoglobin (HAP), and monocyte chemoattractant protein 1 (MCP-1) encoding nucleic acid molecules (*e.g.*, anaphylatoxin receptor (*e.g.*, C3aR and C5aR mRNA) and fragments for use as PCR primers for the amplification or mutation of anaphylatoxin receptor (*e.g.*, C3aR, C5aR, OPN, HAP, MCP-1) nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (*e.g.*, cDNA or genomic DNA) and RNA molecules (*e.g.*, mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

[00165] A nucleic acid molecule used in the methods of the invention, *e.g.*, a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:3 or SEQ ID NO:5 or SEQ ID NO:7 or SEQ ID NO:17, or a portion thereof, can be isolated using standard molecular biology techniques and the sequence information provided herein as well as that known in the art to relate to anaphylatoxin receptors C3aR and C5aR or chronic inflammatory markers OPN, HAP, MCP-1. Using all or portion of the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:3 or SEQ ID NO:5 or SEQ ID NO:7 or SEQ ID NO:17 as a hybridization probe, anaphylatoxin receptor (C3aR, C5aR) or chronic inflammatory markers (OPN, HAP, MCP-1) nucleic acid molecules can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook, J., Fritsh, E.

F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

[00166] Moreover, a nucleic acid molecule encompassing all or a portion of SEQ ID NO:1 or SEQ ID NO:3 or SEQ ID NO: 5 or SEQ ID NO:7 or SEQ ID NO:17 can be isolated by the polymerase chain reaction (PCR) using synthetic oligonucleotide primers designed based upon the sequence of SEQ ID NO:1 or SEQ ID NO:3 or SEQ ID NO: 5 or SEQ ID NO:7 or SEQ ID NO:17.

[00167] A nucleic acid used in the methods of the invention can be amplified using cDNA, mRNA or, alternatively, genomic DNA as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. Furthermore, oligonucleotides corresponding to anaphylatoxin receptor nucleotide sequences can be prepared by standard synthetic techniques, e.g., using an automated DNA synthesizer.

[00168] In a preferred embodiment, the isolated nucleic acid molecules used in the methods of the invention comprise the nucleotide sequence shown in SEQ ID NO:1 or SEQ ID NO:3 or SEQ ID NO: 5 or SEQ ID NO:7 or SEQ ID NO:17, a complement of the nucleotide sequence shown in SEQ ID NO:1 or SEQ ID NO:3 or SEQ ID NO: 5 or SEQ ID NO:7 or SEQ ID NO:17, or a portion of any of these nucleotide sequences. A nucleic acid molecule which is complementary to the nucleotide sequence shown in SEQ ID NO:1 or SEQ ID NO:3 or SEQ ID NO: 5 or SEQ ID NO:7 or SEQ ID NO:17, is one which is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1 or SEQ ID NO:3 or SEQ ID NO: 5 or SEQ ID NO:7 or SEQ ID NO:17 such that it can hybridize to the nucleotide sequence shown in SEQ ID NO:1 or SEQ ID NO:3 or SEQ ID NO: 5 or SEQ ID NO:7 or SEQ ID NO:17 thereby forming a stable duplex.

[00169] Moreover, the nucleic acid molecules used in the methods of the invention can comprise only a portion of the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:3 or SEQ ID NO: 5 or SEQ ID NO:7 or SEQ ID NO:17, for example, a fragment which can be used as a probe or primer or a fragment encoding a portion of an anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammatory marker (e.g., HAP, OPN, MCP-1) protein, e.g., a biologically active portion of an anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammatory marker (e.g., HAP, OPN, MCP-1) protein. The probe or primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12 or 15, preferably about 20 or 25, more preferably about 30, 35, 40, 45, 50, 55, 60, 65, or 75

consecutive nucleotides of a sense sequence of SEQ ID NO:1 or SEQ ID NO:3 or SEQ ID NO: 5 or SEQ ID NO:7 or SEQ ID NO:17 of an anti-sense sequence of SEQ ID NO:1 or SEQ ID NO:3 or SEQ ID NO: 5 or SEQ ID NO:7 or SEQ ID NO:17, or of a naturally occurring allelic variant or mutant of SEQ ID NO:1 or SEQ ID NO:3. In one embodiment, a nucleic acid molecule used in the methods of the invention comprises a nucleotide sequence which is greater than 100, 100-200, 200-300, 300-400, 400-500, 500-600, or more nucleotides in length and hybridizes under stringent hybridization conditions to a nucleic acid molecule of SEQ ID NO:1 or SEQ ID NO:3 or SEQ ID NO: 5 or SEQ ID NO:7 or SEQ ID NO:17.

[00170] As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences that are significantly identical or homologous to each other remain hybridized to each other. Preferably, the conditions are such that sequences at least about 70%, more preferably at least about 80%, even more preferably at least about 85% or 90% identical to each other remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, Ausubel *et al.*, eds., John Wiley & Sons, Inc. (1995), sections 2, 4 and 6. Additional stringent conditions can be found in *Molecular Cloning: A Laboratory Manual*, Sambrook *et al.*, Cold Spring Harbor Press, Cold Spring Harbor, NY (1989), chapters 7, 9 and 11. A preferred, non-limiting example of stringent hybridization conditions includes hybridization in 4X sodium chloride/sodium citrate (SSC), at about 65-70°C (or hybridization in 4X SSC plus 50% formamide at about 42-50°C) followed by one or more washes in 1X SSC, at about 65-70°C. A preferred, non-limiting example of highly stringent hybridization conditions includes hybridization in 1X SSC, at about 65-70°C (or hybridization in 1X SSC plus 50% formamide at about 42-50°C) followed by one or more washes in 0.3X SSC, at about 65-70°C. A preferred, non-limiting example of reduced stringency hybridization conditions includes hybridization in 4X SSC, at about 50-60°C (or alternatively hybridization in 6X SSC plus 50% formamide at about 40-45°C) followed by one or more washes in 2X SSC, at about 50-60°C. Ranges intermediate to the above-recited values, *e.g.*, at 65-70°C or at 42-50°C are also intended to be encompassed by the invention. SSPE (1x SSPE is 0.15M NaCl, 10 mM NaH<sub>2</sub>PO<sub>4</sub>, and 1.25 mM EDTA, pH 7.4) can be substituted for SSC (1x SSC is 0.15M NaCl and 15 mM sodium citrate) in the hybridization and wash buffers; washes are performed for 15 minutes each after hybridization is complete. The hybridization

temperature for hybrids anticipated to be less than 50 base pairs in length should be 5-10°C less than the melting temperature ( $T_m$ ) of the hybrid, where  $T_m$  is determined according to the following equations. For hybrids less than 18 base pairs in length,  $T_m(^{\circ}\text{C}) = 2(\# \text{ of A} + \text{T bases}) + 4(\# \text{ of G} + \text{C bases})$ . For hybrids between 18 and 49 base pairs in length,  $T_m(^{\circ}\text{C}) = 81.5 + 16.6(\log_{10}[\text{Na}^+]) + 0.41(\% \text{G+C}) - (600/N)$ , where N is the number of bases in the hybrid, and  $[\text{Na}^+]$  is the concentration of sodium ions in the hybridization buffer ( $[\text{Na}^+]$  for 1xSSC = 0.165 M). It will also be recognized by the skilled practitioner that additional reagents may be added to hybridization and/or wash buffers to decrease non-specific hybridization of nucleic acid molecules to membranes, for example, nitrocellulose or nylon membranes, including but not limited to blocking agents (e.g., BSA or salmon or herring sperm carrier DNA), detergents (e.g., SDS), chelating agents (e.g., EDTA), Ficoll, PVP and the like. When using nylon membranes, in particular, an additional preferred, non-limiting example of stringent hybridization conditions is hybridization in 0.25-0.5M  $\text{NaH}_2\text{PO}_4$ , 7% SDS at about 65°C, followed by one or more washes at 0.02M  $\text{NaH}_2\text{PO}_4$ , 1% SDS at 65°C, see e.g., Church and Gilbert (1984) *Proc. Natl. Acad. Sci. USA* 81:1991-1995, (or alternatively 0.2X SSC, 1% SDS).

**[00171]** In preferred embodiments, the probe further comprises a label group attached thereto, e.g., the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissue which misexpress an anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammatory marker (e.g., HAP, OPN, MCP-1) protein, such as by measuring a level of an anaphylatoxin receptor-encoding nucleic acid in a sample of cells from a subject e.g., detecting anaphylatoxin receptor (e.g., C3aR, C5aR) mRNA levels or determining whether a genomic anaphylatoxin receptor (e.g., C3aR, C5aR) gene has been mutated or deleted.

**[00172]** The methods of the invention further encompass the use of nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID NO:1 or SEQ ID NO:3 or SEQ ID NO: 5 or SEQ ID NO:7 or SEQ ID NO:17 due to degeneracy of the genetic code and thus encode the same anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammatory marker (e.g., HAP, OPN, MCP-1) proteins as those encoded by the nucleotide sequence shown in SEQ ID NO:1 or SEQ ID NO:3 or SEQ ID NO: 5 or SEQ ID NO:7 or SEQ ID NO:17. In another embodiment, an isolated nucleic acid molecule included in the methods of the invention has a nucleotide sequence encoding a protein having an amino acid

sequence shown in SEQ ID NO:2 or SEQ ID NO:4 or SEQ ID NO: 6 or SEQ ID NO:8 or SEQ ID NO:18.

[00173] The methods of the invention further include the use of allelic variants of human and/or mouse anaphylatoxin receptor, *e.g.*, functional and non-functional allelic variants. Functional allelic variants are naturally occurring amino acid sequence variants of the human and/or mouse anaphylatoxin receptor (*e.g.*, C3aR, C5aR) protein that maintain an anaphylatoxin receptor (*e.g.*, C3aR, C5aR) activity. Functional allelic variants will typically contain only conservative substitution of one or more amino acids of SEQ ID NO:2 or SEQ ID NO:4, or substitution, deletion or insertion of non-critical residues in non-critical regions of the protein.

[00174] Non-functional allelic variants are naturally occurring amino acid sequence variants of the human and/or mouse anaphylatoxin receptor (*e.g.*, C3aR, C5aR) protein that do not have an anaphylatoxin receptor (*e.g.*, C3aR, C5aR) activity. Non-functional allelic variants will typically contain a non-conservative substitution, deletion, or insertion or premature truncation of the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, or a substitution, insertion or deletion in critical residues or critical regions of the protein.

[00175] The methods of the invention may further use non-human orthologues of the human and/or mouse anaphylatoxin receptor (*e.g.*, C3aR, C5aR) protein. Orthologues of the human and/or mouse anaphylatoxin receptor (*e.g.*, C3aR, C5aR) protein are proteins that are isolated from non-human organisms and possess the same anaphylatoxin receptor (*e.g.*, C3aR, C5aR) activity.

[00176] The methods of the invention further include the use of nucleic acid molecules comprising the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:3, or a portion thereof, in which a mutation has been introduced. The mutation may lead to amino acid substitutions at "non-essential" amino acid residues or at "essential" amino acid residues. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of anaphylatoxin receptor (*e.g.*, C3aR, C5aR) (*e.g.*, the sequence of SEQ ID NO:2 or SEQ ID NO:4) without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are conserved among the anaphylatoxin receptor (*e.g.*, C3aR, C5aR) proteins of the invention are not likely to be amenable to alteration.

[00177] Mutations can be introduced into SEQ ID NO:1 or SEQ ID NO:3 by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably,

conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in an anaphylatoxin receptor (*e.g.*, C3aR, C5aR) protein is preferably replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of an anaphylatoxin receptor (*e.g.*, C3aR, C5aR) coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for anaphylatoxin receptor (*e.g.*, C3aR, C5aR) biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO:1 or SEQ ID NO:3, the encoded protein can be expressed recombinantly and the activity of the protein can be determined using the assay described herein.

**[00178]** Another aspect of the invention pertains to the use of isolated nucleic acid molecules which are antisense to the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:3. An "antisense" nucleic acid comprises a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a protein, *e.g.*, complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary to an entire anaphylatoxin receptor (*e.g.*, C3aR, C5aR) coding strand, or to only a portion thereof. In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding an anaphylatoxin receptor. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues. In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding anaphylatoxin receptor. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (also referred to as 5' and 3' untranslated regions).

[00179] Given the coding strand sequences encoding anaphylatoxin receptor (e.g., C3aR, C5aR) disclosed herein, antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of anaphylatoxin receptor (e.g., C3aR, C5aR) mRNA, but more preferably is an oligonucleotide which is antisense to only a portion of the coding or noncoding region of anaphylatoxin receptor (e.g., C3aR, C5aR) mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of anaphylatoxin receptor (e.g., C3aR, C5aR) mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiacytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

**[00180]** The antisense nucleic acid molecules used in the methods of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding an anaphylatoxin receptor (e.g., C3aR, C5aR) protein to thereby inhibit expression of the protein, *e.g.*, by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention include direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, *e.g.*, by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

**[00181]** In yet another embodiment, the antisense nucleic acid molecule used in the methods of the invention is an  $\alpha$ -anomeric nucleic acid molecule. An  $\alpha$ -anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual  $\beta$ -units, the strands run parallel to each other (Gaultier *et al.* (1987) *Nucleic Acids. Res.* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue *et al.* (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue *et al.* (1987) *FEBS Lett.* 215:327-330).

**[00182]** In still another embodiment, an antisense nucleic acid used in the methods of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes (described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave anaphylatoxin receptor (*e.g.*, C3aR, C5aR) mRNA transcripts to thereby inhibit translation of anaphylatoxin receptor (*e.g.*, C3aR, C5aR) mRNA. A ribozyme having specificity for a anaphylatoxin receptor-encoding nucleic acid can be designed based upon the nucleotide sequence of a anaphylatoxin receptor cDNA disclosed herein (*i.e.*, SEQ ID

NO:1 or SEQ ID NO:3). For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a anaphylatoxin receptor-encoding mRNA. See, e.g., U.S. Patent Nos. 4,987,071 and 5,116,742. Alternatively, anaphylatoxin receptor (e.g., C3aR, C5aR) mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, e.g., Bartel, D. and Szostak, J.W. (1993) *Science* 261:1411-1418.

[00183] Alternatively, anaphylatoxin receptor (e.g., C3aR, C5aR) gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the anaphylatoxin receptor (e.g., C3aR, C5aR) (e.g., the anaphylatoxin receptor (e.g., C3aR, C5aR) promoter and/or enhancers) to form triple helical structures that prevent transcription of the anaphylatoxin receptor (e.g., C3aR, C5aR) gene in target cells. See generally, Helene, C. (1991) *Anticancer Drug Des.* 6(6): 569-84; Helene, C. et al. (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher, L.J. (1992) *Bioassays* 14(12):807-15.

[00184] In yet another embodiment, the anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammation marker (e.g., HAP, OPN, MCP-1) nucleic acid molecules used in the methods of the invention can be modified at the base moiety, sugar moiety or phosphate backbone to improve, e.g., the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acid molecules can be modified to generate peptide nucleic acids (see Hyrup B. et al. (1996) *Bioorganic & Medicinal Chemistry* 4:5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, e.g., DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup B. et al. (1996) *supra*; Perry-O'Keefe et al. (1996) *Proc. Natl. Acad. Sci.* 93:14670-675.

[00185] PNAs of anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammation marker (e.g., HAP, OPN, MCP-1) nucleic acid molecules can be used in the therapeutic and diagnostic applications described herein. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, for example, inducing transcription or translation arrest or inhibiting replication. PNAs of anaphylatoxin

receptor (e.g., C3aR, C5aR) or chronic inflammation marker (e.g., HAP, OPN, MCP-1) nucleic acid molecules can also be used in the analysis of single base pair mutations in a gene, (e.g., by PNA-directed PCR clamping); as 'artificial restriction enzymes' when used in combination with other enzymes, (e.g., S1 nucleases (Hyrup B. *et al.* (1996) *supra*)); or as probes or primers for DNA sequencing or hybridization (Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *et al.* (1996) *supra*).

[00186] In another embodiment, PNAs of anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammation marker (e.g., HAP, OPN, MCP-1) can be modified, (e.g., to enhance their stability or cellular uptake), by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammation marker (HAP, OPN, MCP-1) nucleic acid molecules can be generated which may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, (e.g., RNase H and DNA polymerases), to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (Hyrup B. *et al.* (1996) *supra*). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup B. *et al.* (1996) *supra* and Finn P.J. *et al.* (1996) *Nucleic Acids Res.* 24 (17): 3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry and modified nucleoside analogs, e.g., 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used as a between the PNA and the 5' end of DNA (Mag, M. *et al.* (1989) *Nucleic Acid Res.* 17: 5973-88). PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5'PNA segment and a 3'DNA segment (Finn P.J. *et al.* (1996) *supra*). Alternatively, chimeric molecules can be synthesized with a 5'DNA segment and a 3'PNA segment (Peterser, K.H. *et al.* (1975) *Bioorganic Med. Chem. Lett.* 5: 1119-1124).

[00187] In other embodiments, the oligonucleotide used in the methods of the invention may include other appended groups such as peptides (e.g., for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, e.g., Letsinger *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:6553-6556; Lemaitre *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:648-652; PCT Publication No. W088/09810) or the blood-brain barrier (see, e.g., PCT Publication No. W089/10134). In addition, oligonucleotides can be modified with

hybridization-triggered cleavage agents (See, e.g., Krol *et al.* (1988) *Bio-Techniques* 6:958-976) or intercalating agents. (See, e.g., Zon (1988) *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, (e.g., a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent).

Isolated anaphylatoxin receptor proteins and

Anti-anaphylatoxin receptor Antibodies Used in the Methods of the Invention

[00188] The methods of the invention include the use of isolated anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammation marker (e.g., HAP, OPN, MCP-1) proteins, and biologically active portions thereof, as well as polypeptide fragments suitable for use as immunogens to raise anti-anaphylatoxin receptor (e.g., C3aR, C5aR) or anti-chronic inflammation marker (e.g., HAP, OPN, MCP-1) antibodies. In one embodiment, native anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammation marker (e.g., HAP, OPN, MCP-1) proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammation marker (e.g., HAP, OPN, MCP-1) proteins are produced by recombinant DNA techniques. Alternative to recombinant expression, an anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammation marker (e.g., HAP, OPN, MCP-1) protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

[00189] As used herein, a "biologically active portion" of an anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammation marker (e.g., HAP, OPN, MCP-1) protein includes a fragment of an anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammation marker (e.g., HAP, OPN, MCP-1) protein having an anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammation marker (e.g., HAP, OPN, MCP-1) activity. Biologically active portions of an anaphylatoxin receptor (e.g., C3aR, C5aR) protein include peptides comprising amino acid sequences sufficiently identical to or derived from the amino acid sequence of the anaphylatoxin receptor (e.g., C3aR, C5aR) protein, e.g., the amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:4, which include fewer amino acids than the full length anaphylatoxin receptor (e.g., C3aR, C5aR) proteins, and exhibit at least one activity of a anaphylatoxin receptor (e.g., C3aR, C5aR) protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the anaphylatoxin receptor (e.g., C3aR, C5aR) protein (e.g., the N-terminal region of the anaphylatoxin

receptor (e.g., C3aR, C5aR) protein that is believed to be involved in the regulation of apoptotic activity). A biologically active portion of an anaphylatoxin receptor (e.g., C3aR, C5aR) protein can be a polypeptide which is, for example, 25, 50, 75, 100, 125, 150, 175, 200, 250, 300 or more amino acids in length. Biologically active portions of an anaphylatoxin receptor (e.g., C3aR, C5aR) protein can be used as targets for developing agents which modulate an anaphylatoxin receptor (e.g., C3aR, C5aR) activity.

**[00190]** In a preferred embodiment, the anaphylatoxin receptor (e.g., C3aR, C5aR) protein used in the methods of the invention has an amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:4. In other embodiments, the anaphylatoxin receptor (e.g., C3aR, C5aR) protein is substantially identical to SEQ ID NO:2 or SEQ ID NO:4, and retains the functional activity of the protein of SEQ ID NO:2 or SEQ ID NO:4 yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail above.

**[00191]** In a preferred embodiment, the chronic inflammation marker (e.g., HAP, OPN, MCP-1) protein used in the methods of the invention has an amino acid sequence shown in SEQ ID NO:6 or SEQ ID NO:8 or SEQ ID NO:18. In other embodiments, the chronic inflammation marker (e.g., HAP, OPN, MCP-1) protein is substantially identical to SEQ ID NO:6 or SEQ ID NO:8 or SEQ ID NO:18, and retains the functional activity of the protein of SEQ ID NO:6 or SEQ ID NO:8 or SEQ ID NO:18 yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail above.

**[00192]** To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-identical sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, or 90% of the length of the reference sequence (e.g., when aligning a second sequence to the anaphylatoxin receptor (e.g., C3aR, C5aR) amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The

percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

[00193] The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* 48:444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at <http://www.gcg.com>), using either a Blosum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at <http://www.gcg.com>), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. In another embodiment, the percent identity between two amino acid or nucleotide sequences is determined using the algorithm of E. Meyers and W. Miller (*Comput. Appl. Biosci.* 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0 or 2.0U), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

[00194] The methods of the invention may also use anaphylatoxin receptor (e.g., C3aR, C5aR) chimeric or fusion proteins. As used herein, an anaphylatoxin receptor (e.g., C3aR, C5aR) "chimeric protein" or "fusion protein" comprises a anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide operatively linked to a non-anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide. An "anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide" refers to a polypeptide having an amino acid sequence corresponding to an anaphylatoxin receptor (e.g., C3aR, C5aR) molecule, whereas a "non-anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein which is not substantially homologous to the anaphylatoxin receptor (e.g., C3aR, C5aR) protein, e.g., a protein which is different from the anaphylatoxin receptor (e.g., C3aR, C5aR) protein and which is derived from the same or a different organism. Within an anaphylatoxin receptor (e.g., C3aR, C5aR) fusion protein the anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide can correspond to all or a portion of an anaphylatoxin receptor (e.g., C3aR, C5aR) protein. In a preferred embodiment, an anaphylatoxin receptor (e.g., C3aR, C5aR) fusion protein comprises at least one biologically active portion of an

anaphylatoxin receptor (e.g., C3aR, C5aR) protein. In another preferred embodiment, an anaphylatoxin receptor/fusion protein comprises at least two biologically active portions of an anaphylatoxin receptor (e.g., C3aR, C5aR) protein. Within the fusion protein, the term "operatively linked" is intended to indicate that the anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide and the non-anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide are fused in-frame to each other. The non-anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide can be fused to the N-terminus or C-terminus of the anaphylatoxin receptor (e.g., C3aR, C5aR) polypeptide.

[00195] For example, in one embodiment, the fusion protein is a GST-anaphylatoxin receptor (e.g., C3aR, C5aR) fusion protein in which the anaphylatoxin receptor (e.g., C3aR, C5aR) sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant anaphylatoxin receptor.

[00196] In another embodiment, this fusion protein is a anaphylatoxin receptor (e.g., C3aR, C5aR) protein containing a heterologous signal sequence at its N-terminus. In certain host cells (e.g., mammalian host cells), expression and/or secretion of anaphylatoxin receptor (e.g., C3aR, C5aR) can be increased through use of a heterologous signal sequence.

[00197] The anaphylatoxin receptor (e.g., C3aR, C5aR) fusion proteins used in the methods of the invention can be incorporated into pharmaceutical compositions and administered to a subject *in vivo*. The anaphylatoxin receptor (e.g., C3aR, C5aR) fusion proteins can be used to affect the bioavailability of an anaphylatoxin receptor (e.g., C3aR, C5aR) substrate. Use of anaphylatoxin receptor (e.g., C3aR, C5aR) fusion proteins may be useful therapeutically for the treatment of disorders caused by, for example, (i) aberrant modification or mutation of a gene encoding an anaphylatoxin receptor protein; (ii) mis-regulation of the anaphylatoxin receptor (e.g., C3aR, C5aR) gene; and (iii) aberrant post-translational modification of an anaphylatoxin receptor (e.g., C3aR, C5aR) protein.

[00198] Moreover, the anaphylatoxin receptor-fusion proteins used in the methods of the invention can be used as immunogens to produce anti-anaphylatoxin receptor (e.g., C3aR, C5aR) antibodies in a subject, to purify anaphylatoxin receptor (e.g., C3aR, C5aR) ligands and in screening assays to identify molecules which inhibit the interaction of anaphylatoxin receptor (e.g., C3aR, C5aR) with an anaphylatoxin receptor (e.g., C3aR, C5aR) substrate.

[00199] Preferably, an anaphylatoxin receptor (e.g., C3aR, C5aR) chimeric or fusion protein used in the methods of the invention is produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences

are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, *Current Protocols in Molecular Biology*, eds. Ausubel *et al.* John Wiley & Sons: 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (*e.g.*, a GST polypeptide). An anaphylatoxin receptor-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the anaphylatoxin receptor (*e.g.*, C3aR, C5aR) protein.

**[00200]** The invention also pertains to the use of variants of the anaphylatoxin receptor (*e.g.*, C3aR, C5aR) proteins which function as either anaphylatoxin receptor (*e.g.*, C3aR, C5aR) activators (mimetics) or as anaphylatoxin receptor (*e.g.*, C3aR, C5aR) inhibitors. Variants of the anaphylatoxin receptor (*e.g.*, C3aR, C5aR) proteins can be generated by mutagenesis, *e.g.*, discrete point mutation or truncation of a anaphylatoxin receptor (*e.g.*, C3aR, C5aR) protein. An agonist of the anaphylatoxin receptor (*e.g.*, C3aR, C5aR) proteins can retain substantially the same, or a subset, of the biological activities of the naturally occurring form of an anaphylatoxin receptor (*e.g.*, C3aR, C5aR) protein. An inhibitor of an anaphylatoxin receptor (*e.g.*, C3aR, C5aR) protein can inhibit one or more of the activities of the naturally occurring form of the anaphylatoxin receptor (*e.g.*, C3aR, C5aR) protein by, for example, competitively modulating an anaphylatoxin receptor-mediated activity of an anaphylatoxin receptor (*e.g.*, C3aR, C5aR) protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the anaphylatoxin receptor (*e.g.*, C3aR, C5aR) protein.

**[00201]** In one embodiment, variants of an anaphylatoxin receptor (*e.g.*, C3aR, C5aR) protein which function as either anaphylatoxin receptor (*e.g.*, C3aR, C5aR) activators (mimetics) or as anaphylatoxin receptor (*e.g.*, C3aR, C5aR) inhibitors can be identified by

screening combinatorial libraries of mutants, *e.g.*, truncation mutants, of an anaphylatoxin receptor (*e.g.*, C3aR, C5aR) protein for anaphylatoxin receptor (*e.g.*, C3aR, C5aR) protein activator or inhibitor activity. In one embodiment, a variegated library of anaphylatoxin receptor (*e.g.*, C3aR, C5aR) variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of anaphylatoxin receptor (*e.g.*, C3aR, C5aR) variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential anaphylatoxin receptor (*e.g.*, C3aR, C5aR) sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage display) containing the set of anaphylatoxin receptor (*e.g.*, C3aR, C5aR) sequences therein. There are a variety of methods which can be used to produce libraries of potential anaphylatoxin receptor (*e.g.*, C3aR, C5aR) variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential anaphylatoxin receptor (*e.g.*, C3aR, C5aR) sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, *e.g.*, Narang, S.A. (1983) *Tetrahedron* 39:3; Itakura *et al.* (1984) *Annu. Rev. Biochem.* 53:323; Itakura *et al.* (1984) *Science* 198:1056; Ike *et al.* (1983) *Nucleic Acid Res.* 11:477).

[00202] In addition, libraries of fragments of an anaphylatoxin receptor (*e.g.*, C3aR, C5aR) protein coding sequence can be used to generate a variegated population of anaphylatoxin receptor (*e.g.*, C3aR, C5aR) fragments for screening and subsequent selection of variants of an anaphylatoxin receptor (*e.g.*, C3aR, C5aR) protein. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of an anaphylatoxin receptor (*e.g.*, C3aR, C5aR) coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA which can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal, C-terminal and internal fragments of various sizes of the anaphylatoxin receptor (*e.g.*, C3aR, C5aR) protein.

[00203] Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of anaphylatoxin receptor (e.g., C3aR, C5aR) proteins. The most widely used techniques, which are amenable to high through-put analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a new technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify anaphylatoxin receptor (e.g., C3aR, C5aR) variants (Arkin and Yourvan (1992) *Proc. Natl. Acad. Sci. USA* 89:7811-7815; Delgrave *et al.* (1993) *Protein Engineering* 6(3):327-331).

[00204] The methods of the invention further include the use of anti-anaphylatoxin receptor (e.g., C3aR, C5aR) or anti-chronic inflammation marker (e.g., HAP, OPN, MCP-1) antibodies. An isolated anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammation marker (e.g., HAP, OPN, MCP-1) protein, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that bind anaphylatoxin receptor (e.g., C3aR, C5aR) or a chronic inflammation marker (e.g., HAP, OPN, MCP-1) using standard techniques for polyclonal and monoclonal antibody preparation. A full-length anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammation marker (e.g., HAP, OPN, MCP-1) protein can be used or, alternatively, antigenic peptide fragments of anaphylatoxin receptor (e.g., C3aR, C5aR) or a chronic inflammation marker (e.g., HAP, OPN, MCP-1) can be used as immunogens. The antigenic peptide of anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammation marker (e.g., HAP, OPN, MCP-1) comprises at least 8 amino acid residues of the amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:4 or SEQ ID NO:6 or SEQ ID NO:8 or SEQ ID NO:18 and encompasses an epitope of anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammation marker (e.g., HAP, OPN, MCP-1) such that an antibody raised against the peptide forms a specific immune complex with the anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammation marker (e.g., HAP, OPN, MCP-1) protein. Preferably, the antigenic peptide comprises at least 10 amino acid

residues, more preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues.

[00205] Preferred epitopes encompassed by the antigenic peptide are regions of anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammation marker (e.g., HAP, OPN, MCP-1) that are located on the surface of the protein, *e.g.*, hydrophilic regions, as well as regions with high antigenicity.

[00206] An anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammation marker (e.g., HAP, OPN, MCP-1) immunogen is typically used to prepare antibodies by immunizing a suitable subject, (*e.g.*, rabbit, goat, mouse, or other mammal) with the immunogen. An appropriate immunogenic preparation can contain, for example, recombinantly expressed anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammation marker (e.g., HAP, OPN, MCP-1) protein or a chemically synthesized anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammation marker (e.g., HAP, OPN, MCP-1) polypeptide. The preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or similar immunostimulatory agent. Immunization of a suitable subject with an immunogenic anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammation marker (e.g., HAP, OPN, MCP-1) preparation induces a polyclonal anti-anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammation marker (e.g., HAP, OPN, MCP-1) antibody response.

[00207] The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site which specifically binds (immunoreacts with) an antigen, such as a C3aR or C5aR or HAP or OPN or MCP-1. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and F(ab')<sub>2</sub> fragments which can be generated by treating the antibody with an enzyme such as pepsin. The invention provides polyclonal and monoclonal antibodies that bind anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammation marker (e.g., HAP, OPN, MCP-1) molecules. The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of anaphylatoxin receptor. A monoclonal antibody composition thus typically displays a single binding affinity for a particular anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammation marker (e.g., HAP, OPN, MCP-1) protein with which it immunoreacts.

**[00208]** Polyclonal anti-anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammation marker (e.g., HAP, OPN, MCP-1) antibodies can be prepared as described above by immunizing a suitable subject with a anaphylatoxin receptor immunogen. The anti-anaphylatoxin receptor (e.g., C3aR, C5aR) or anti-chronic inflammation marker (e.g., HAP, OPN, MCP-1) antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized anaphylatoxin receptor. If desired, the antibody molecules directed against anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammation marker (e.g., HAP, OPN, MCP-1) can be isolated from the mammal (e.g., from the blood) and further purified by well known techniques, such as protein A chromatography to obtain the IgG fraction. At an appropriate time after immunization, e.g., when the anti-anaphylatoxin receptor (e.g., C3aR, C5aR) or anti- chronic inflammation marker (e.g., HAP, OPN, MCP-1) antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) *Nature* 256:495-497) (see also, Brown *et al.* (1981) *J. Immunol.* 127:539-46; Brown *et al.* (1980) *J. Biol. Chem.* 255:4980-83; Yeh *et al.* (1976) *Proc. Natl. Acad. Sci. USA* 73:2927-31; and Yeh *et al.* (1982) *Int. J. Cancer* 29:269-75), the more recent human B cell hybridoma technique (Kozbor *et al.* (1983) *Immunol Today* 4:72), the EBV-hybridoma technique (Cole *et al.* (1985) *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing monoclonal antibody hybridomas is well known (see generally Kenneth, R. H. in *Monoclonal Antibodies: A New Dimension In Biological Analyses*, Plenum Publishing Corp., New York, New York (1980); Lerner, E. A. (1981) *Yale J. Biol. Med.* 54:387-402; Gefter, M. L. *et al.* (1977) *Somatic Cell Genet.* 3:231-36). Briefly, an immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with an anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammation marker (e.g., HAP, OPN, MCP-1) immunogen as described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds anaphylatoxin receptor or a chronic inflammation marker.

**[00209]** Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating an anti-anaphylatoxin receptor (e.g., C3aR, C5aR) or anti-chronic inflammation marker (e.g., HAP, OPN, MCP-1)

monoclonal antibody (see, e.g., G. Galfre *et al.* (1977) *Nature* 266:55052; Gefter *et al.* (1977) *supra*; Lerner (1981) *supra*; and Kenneth (1980) *supra*). Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods which also would be useful. Typically, the immortal cell line (e.g., a myeloma cell line) is derived from the same mammalian species as the lymphocytes. For example, murine hybridomas can be made by fusing lymphocytes from a mouse immunized with an immunogenic preparation of the invention with an immortalized mouse cell line. Preferred immortal cell lines are mouse myeloma cell lines that are sensitive to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium"). Any of a number of myeloma cell lines can be used as a fusion partner according to standard techniques, e.g., the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from ATCC. Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using polyethylene glycol ("PEG"). Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridoma cells producing a monoclonal antibody of the invention are detected by screening the hybridoma culture supernatants for antibodies that bind anaphylatoxin receptor, e.g., using a standard ELISA assay.

[00210] Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal anti-anaphylatoxin receptor (e.g., C3aR, C5aR) or anti-chronic inflammation marker (e.g., HAP, OPN, MCP-1) antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (e.g., an antibody phage display library) with anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammation marker (e.g., HAP, OPN, MCP-1) to thereby isolate immunoglobulin library members that bind anaphylatoxin receptor or a chronic inflammation marker. Kits for generating and screening phage display libraries are commercially available (e.g., the Pharmacia *Recombinant Phage Antibody System*, Catalog No. 27-9400-01; and the Stratagene *SurfZAP™ Phage Display Kit*, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, U.S. Patent No. 5,223,409; PCT Publication No. WO 92/18619; PCT Publication No. WO 91/17271; PCT Publication No. WO 92/20791; PCT Publication No. WO 92/15679; PCT Publication No. WO 93/01288; PCT Publication No. WO 92/01047; PCT Publication No. WO 92/09690; PCT Publication No. WO 90/02809; Fuchs *et al.* (1991) *Bio/Technology*

9:1370-1372; Hay *et al.* (1992) *Hum. Antibod. Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281; Griffiths *et al.* (1993) *EMBO J* 12:725-734; Hawkins *et al.* (1992) *J. Mol. Biol.* 226:889-896; Clarkson *et al.* (1991) *Nature* 352:624-628; Gram *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:3576-3580; Garrad *et al.* (1991) *Bio/Technology* 9:1373-1377; Hoogenboom *et al.* (1991) *Nuc. Acid Res.* 19:4133-4137; Barbas *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:7978-7982; and McCafferty *et al.* (1990) *Nature* 348:552-554.

[00211] Additionally, recombinant anti-anaphylatoxin receptor (e.g., C3aR, C5aR) or anti-chronic inflammation marker (e.g., HAP, OPN, MCP-1) antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the methods of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in Robinson *et al.* International Application No. PCT/US86/02269; Akira, *et al.* European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison *et al.* European Patent Application 173,494; Neuberger *et al.* PCT Publication No. WO 86/01533; U.S. Patent No. 4,816,567; Cabilly *et al.* European Patent 125,023; Better *et al.* (1988) *Science* 240:1041-1043; Liu *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:3439-3443; Liu *et al.* (1987) *J. Immunol.* 139:3521-3526; Sun *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:214-218; Nishimura *et al.* (1987) *Canc. Res.* 47:999-1005; Wood *et al.* (1985) *Nature* 314:446-449; Shaw *et al.* (1988) *J. Natl. Cancer Inst.* 80:1553-1559; Morrison, S. L. (1985) *Science* 229:1202-1207; Oi *et al.* (1986) *BioTechniques* 4:214; U.S. Patent 5,225,539; Jones *et al.* (1986) *Nature* 321:552-525; Verhoeven *et al.* (1988) *Science* 239:1534; and Beidler *et al.* (1988) *J. Immunol.* 141:4053-4060.

[00212] An anti-anaphylatoxin receptor (e.g., C3aR, C5aR) or anti-chronic inflammation marker (e.g., HAP, OPN, MCP-1) antibody can be used to detect anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammation marker (e.g., HAP, OPN, MCP-1) protein (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammation marker (e.g., HAP, OPN, MCP-1) protein. Anti-anaphylatoxin receptor (e.g., C3aR, C5aR) or chronic inflammation marker (e.g., HAP, OPN, MCP-1) antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, e.g., to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance.

Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials.

Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase,  $\beta$ -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{35}\text{S}$  or  $^3\text{H}$ .

**[00213]** This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application, as well as the Sequence Listing is incorporated herein by reference.

#### EXEMPLIFICATION

Example 1: Anaphylatoxin Receptors C3aR and C5aR Gene Expression In Human Tissues

**[00214]** Expression of the anaphylatoxin receptor (C3aR and C5aR) transcripts were quantitated in human tissues using real-time quantitative RT-PCR. Tissue samples included the following normal human tissues: pancreas, spleen, small intestine, kidney, liver, heart, brain, lung, placenta, trachea, skeletal muscle, adrenal gland, testis, thymus, adipose, stomach, brain(hypothalamus), and macrophages.

**[00215]** Results demonstrated that the C3aR transcript is found expressed at highest levels in macrophages, followed by spleen, placenta, adipose, and small intestine. Lower expression was detected in brain, adrenal gland, trachea, liver, kidney, lung, testis, thymus, stomach, and hypothalamus.

**[00216]** Results demonstrated that the C5aR transcript is found expressed at highest levels in spleen, followed by adipose, macrophages, and lung. Lower expression was detected in trachea, placenta, small intestine, adrenal gland, testis, and thymus.

**TABLE 1: Human C3aR and C5aR Expression**

Tissue	C3aR	C5aR
Pancreas	1	1
Spleen	94.4	115
Small Intestine	61.4	13.7
Liver	15.4	4.2
Kidney	10.9	4.5
Heart	2.4	1.9
Brain	23.3	2.7
Lung	8.8	13.3
Placenta	79.9	11.1
Trachea	18.7	18.5
Skeletal Muscle	2.3	1.1
Adrenal gland	20.1	11.1
Testis	12.2	6.1
Thymus	11.4	5.5
Fat	62.5	40.6
Stomach	17.3	1.6
Hypothalamus	7.1	1.8
Macrophage	133.4	27.4

**[00217]** Total RNA was prepared using the trizol method and treated with DNase to remove contaminating genomic DNA. cDNA was synthesized using standard techniques. Mock cDNA synthesis in the absence of reverse transcriptase resulted in samples with no detectable PCR amplification of the control 18S gene, confirming efficient removal of genomic DNA contamination. Anaphylatoxin receptor (C3aR or C5aR) expression was measured by TaqMan quantitative PCR analysis, performed according to the manufacturer's directions (Perkin Elmer Applied Biosystems, Foster City, CA).

**[00218]** PCR probes were designed by PrimerExpress software (Perkin Elmer Applied Biosystems) based on the human anaphylatoxin receptor (e.g., C3aR, C5aR) sequence. To standardize the results between the different tissues, two probes, distinguished by different fluorescent labels, were added to each sample. The differential labeling of the probe for the anaphylatoxin receptor (C3aR or C5aR) and the probe for 18S RNA (as an internal control)

thus enabled their simultaneous measurement in the same well. Forward and reverse primers and the probes for both 18S RNA and human or murine anaphylatoxin receptor (C3aR or C5aR) were added to the TaqMan Universal PCR Master Mix (PE Applied Biosystems). Although the final concentration of primer and probe could vary, each was internally consistent within a given experiment. A typical experiment contained 200 nM each of the forward and reverse primers and 100 nM of the probe for the 18S RNA, as well as 600 nM of each of the forward and reverse primers and 200 nM of the probe for the anaphylatoxin receptor (C3aR or C5aR). TaqMan matrix experiments were carried out using an ABI PRISM 770 Sequence Detection System (PE Applied Biosystems). The thermal cycler conditions were as follows: hold for 2 minutes at 50°C and 10 minutes at 95°C, followed by two-step PCR for 40 cycles of 95°C for 15 seconds, followed by 60°C for 1 minute.

**[00219]** The following method was used to quantitatively calculate anaphylatoxin receptor (C3aR or C5aR) gene expression in the tissue samples, relative to the 18S RNA expression in the same tissue. The threshold values at which the PCR amplification started were determined using the manufacturer's software. PCR cycle number at threshold value was designated as CT. Relative expression was calculated as  $2^{-(CT_{\text{test}} - CT_{18S})}$  tissue of interest -  $(CT_{\text{test}} - CT_{18S})$  lowest expressing tissue in panel. Samples were run in duplicate and the averages of 2 relative expression levels that were linear to the amount of template cDNA with a slope similar to the slope for the internal control 18S were used.

#### Example 2: Anaphylatoxin Receptors C3aR and C5aR Gene Expression In Mouse Tissues

**[00220]** Expression of the anaphylatoxin receptor (e.g., C3aR, C5aR) transcripts were quantitated in mouse tissues using real-time quantitative RT-PCR as described above. Normal mouse tissues examined included the following: pancreas, white fat (WAT), brown fat (BAT), liver, spleen, stomach, tongue, heart, hypothalamus, brain, kidney, testis, intestine, muscle, lung, macrophages, stromal-vascular cells, and adipocytes. The latter two samples were isolated from epididymal white fat of normal mice.

**[00221]** The results demonstrated that the C3aR transcript is found expressed at highest levels in macrophages and stromal-vascular cells, as well as whole adipose tissue, and significantly lower expression in the tongue, heart, intestine, lung and brown fat.

**[00222]** The results demonstrated that the C5aR transcript is found expressed at highest levels in stromal-vascular cells and macrophages, followed by white fat, and significantly lower expression in the lung, adipose tissue, heart, tongue, and brown fat.

**TABLE 2: Murine C3aR and C5aR Expression**

Tissue	C3aR	C5aR
<b>Pancreas</b>	1	1
<b>WAT</b>	18.7	256
<b>BAT</b>	2.8	27.2
<b>Liver</b>	0.7	3.1
<b>Spleen</b>	0.5	18.0
<b>Stomach</b>	1.1	16.5
<b>Tongue</b>	3.3	34.9
<b>Heart</b>	3.0	40.6
<b>Hypothalamus</b>	1.0	4.0
<b>Brain</b>	1.6	4.4
<b>Kidney</b>	0.9	5.3
<b>Testis</b>	1.4	4.3
<b>Intestine</b>	4.3	12.3
<b>Muscle</b>	0.9	8.5
<b>Lung</b>	4.5	81.9
<b>Macrophage</b>	1184.3	828.9
<b>Stromal-Vascular</b>	309.2	1423.3
<b>Adipocytes</b>	9.9	59.1

Example 3: Regulation of anaphylatoxin receptors C3aR and C5aR in genetic models of obesity and diabetes.

**[0207]** Tissues of genetic mouse models of obesity (*ob/ob* and *db/db*) were also examined for anaphylatoxin receptors C3aR and C5aR expression (including white fat, lung, spleen, macrophage). Detection of transcripts was measured as described above. The results indicate that the anaphylatoxin receptors C3aR and C5aR transcripts are expressed in white fat at higher levels in the *ob/ob* and *db/db* mouse relative to the expression levels in wildtype mouse. No significant regulation was seen in additional tissues analyzed. C3aR is expressed at significantly higher levels in *ob/ob* mice and in *db/db* mice than wild-type

mice. See Table 3. Additionally, C5aR is expressed at significantly higher levels in ob/ob mice and in db/db mice than wild type mice. See Table 3. Still further, upregulation of both C3aR and C5aR in ob/ob and db/db mice is specific to increased expression in adipose tissue.

**TABLE 3: Expression of C3aR and C5aR  
in Genetic Models of Obesity and Diabetes**

	<b>Relative expression WAT</b>	
<b>genotype</b>	C3aR	C5aR
<b>wild type</b>	1	1
<b>ob/ob</b>	5.9	5
<b>wild type</b>	1	1
<b>db/db</b>	3.2	3.4

Example 4: Regulation of anaphylatoxin receptors C3aR and C5aR in a diet-induced obesity model.

[0208] Expression of anaphylatoxin receptors C3aR and C5aR was also examined in a diet induced obesity mouse model system. Briefly, wild-type C57BL/6J (The Jackson Laboratory, Bar Harbor, Maine) mice on diets containing 10% or 60% kcal from fat (Research Diets, New Brunswick, NJ). These mice were started on diets at 4-5 weeks of age. Mice were sacrificed for tissue collection at 20 weeks of age. Detection of transcripts were measured using real time PCR as described above. The results indicate that the anaphylatoxin receptors C3aR and C5aR transcripts are both expressed at significantly higher levels in white adipose tissue (WAT) in mice maintained on a high fat diet. See Table 4.

**TABLE 4: Expression of C3aR and C5aR  
in Diet Induced Obesity Models**

	<b>Relative expression WAT</b>	
<b>diet</b>	C3aR	C5aR
<b>Control</b>	1	1
<b>High Fat</b>	14.5	6.7

Example 5: Separation of WAT into Stromal-vascular fraction and adipocytes

**[0209]** Fat tissue was separated into stromal-vascular fractions and adipocytes to examine localization of anaphylatoxin receptors C3aR and C5aR expression. Briefly, epididymal fat pads were excised from C57BL/6J male mice, weighed and rinsed in isolation buffer (solution containing 1x KRP, 20mM Hepes, 200nM adenosine and 2.5% BSA, PH 7.5, kept at 37°C). Fat pads were cut into tiny pieces in digestion buffer (isolation buffer supplemented with collagenase 1mg/ml, 2mg/g of tissue) and incubated at 37°C in shaking water bath at 100 rpm/min for one hour. The digested tissue was filtered through 400µM mesh (TETKO, location) covered on cut-bottom syringes to obtain a single cell suspension. Stromal-vascular cells and adipocytes were separated after centrifuging at 1000rpm for 2 minutes at room temperature, with adipocytes floating on the surface of isolation buffer and form a white layer. Adipocytes were transferred to clean tubes and washed twice with the isolation buffer before Trizol solution was added for RNA extraction. The pellet containing stromal-vascular cells was re-suspended in the red blood cell lysis buffer (0.83% NH<sub>4</sub>Cl, 0.05mM Na<sub>2</sub>EDTA, 0.1% KHCO<sub>3</sub>, pH 7.3) to dissolve the red blood cells and was then spun down for RNA extraction as described above. Detection of transcripts was measured using real time PCR as described above. Results indication that C3aR and C5aR are predominantly expressed in the stromal-vascular fraction of fat tissue as compared to adipocytes. See Table 5. Conversely, leptin demonstrates predominant expression in adipocytes. In situ hybridization and immunohistochemistry experiments using stromal-vascular fractions confirmed C3aR is expressed in macrophages in stromal vascular fractions of both wild type and ob/ob animals.

**TABLE 5: Expression of C3aR and C5aR is Localized to Stomal-Vascular Fraction of Fat**

<b>Tissue</b>	<b>Relative expression</b>		
	<b>Leptin</b>	<b>C3aR</b>	<b>C5aR</b>
<b>SV</b>	1	30.9	12.8
<b>ADI</b>	29.7	1	1

Example 6: Inflammation Markers Predict the Degree of Severity of Insulin Resistance

**[0210]** Mice were placed on regular chow diet and high fat diet as described above. Mice were sacrificed at time intervals on respective diets, and tissues and fluid analyzed.

RNA was extracted from tissue and serum, and analyzed using real time PCR as described above.

[0211] Results demonstrated upregulation, over time, of leukocyte and inflammation related genes haptoglobin (HAP) macrophage chemotactic protein-1 (MCP-1) and osteopontin (OPN) in adipose tissue in mice exposed to a high fat diet. See Table 6. Further, the upregulation correlated with degree of insulin resistance over time, as determined by plasma insulin levels. Similar results in upregulation of these inflammation related genes was found in mouse models of obesity and diabetes. Closer analysis of the cell types responsible for the expression levels seen in WAT was done by separation of WAT into stromal-vascular fraction and adipocytes as described above in Example 5. This analysis demonstrated expression of HAP is predominantly from adipocytes but is abundant the stroma as well. MCP-1, on the other hand, originates primarily from the stromal-vascular material (data not shown).

**TABLE 6: Expression of Haptoglobin, Macrophage Chemotactic Protein-1 and Osteopontin are Upregulated as Insulin Resistance Progresses**

<u>Time (weeks)</u>	<b>Relative Expression</b>					
	<b>HAP</b>		<b>MCP-1</b>		<b>OPN</b>	
	<b>Control</b>	<b>High fat</b>	<b>Control</b>	<b>High fat</b>	<b>Control</b>	<b>High fat</b>
<b>0</b>	1.0		1.0		1.0	
<b>6</b>	1.7	1.9	3.2	8.8	1.7	6.5
<b>16</b>	1.1	4.2	1.8	24.3	1.7	65.1
<b>26</b>	1.6	5.1	4.8	29.4	2.8	78.2

#### Example 7: Inflammation Marker Levels in Obese Mice

[0212] Tissues of genetic mouse models of obesity (*ob/ob* and *db/db*) were also examined for expression of HAP and MCP-1. RNA was extracted, and analyzed using real time PCR as described above from wild type, *ob/ob*, and *db/db* mice. Taqman analysis revealed that the transcripts of both genes are clearly up-regulated in WAT from both rodent obesity models.

#### Example 8: Serum Protein Levels of Inflammation Markers

[0213] The MCP-1 and HAP genes were chosen for further study due to the fact that they are readily detectable in serum. Serum MCP-1 was quantitated by EIA, elisa immunoblot assay (R&D Systems, Minneapolis, MN). We found that MCP-1 levels in ob/ob mice are significantly higher than those of controls (see Table 7).

[0214] Since an elisa immunoblot assay to detect rodent HAP is not available, we performed a qualitative Western blot analysis of mouse serum. Qualitative Western blot analysis of HAP was performed by separating 0.2 uL of serum by SDS-PAGE and transferring to a nylon membrane. The membrane was incubated with a sheep anti-haptoglobin antibody (Ab) (Biodesign, Saco, ME) and visualized with an HRP conjugated anti-sheep IgG Ab (Santa Cruz Biotechnology, Santa Cruz, CA) and ECL western detection reagents (Amersham Biosciences, Piscataway, NJ). Serum HAP was significantly upregulated in ob/ob mice that in controls (data not shown).

**TABLE 7: Expression of MCP-1 is Upregulated  
in Obese Mice**

<u>genotype</u>	<u>MCP-1 (pg/mL)</u>
WT	91.9
ob/ob	410

[0215] In addition, serum protein levels of MCP-1 and HAP were measured in wild type mice exposed to a high fat diet, as they progress toward obesity and insulin resistance. The experiments were carried out as described in example 6, with serum protein levels measured at specified time point. Levels of both gene products for MCP-1 and HAP rose consistent with levels found in mRNA analysis described above (data not shown).

#### Example 9: Treatment with Thiazolidinones

[0216] Further evidence for a role of the inflammatory proteins in insulin resistance was demonstrated by the results of experiments with anti-diabetic therapies in ob/ob mice. Thiazolidinones (TZDs), a class of insulin sensitizing drugs, work by agonizing PPAR $\gamma$ . TZDs have also been shown to have anti-inflammatory activity. Rosiglitazone, a member of the TZDs, was used for treatment of genetically diabetic ob/ob mice. For rosiglitazone treatment, 8 week old *ob/ob* male mice from Jackson were acclimated for one week.

Rosiglitazone or vehicle (sterile water) was orally gavaged once a day at a dose of 15 mg/kg for 28 consecutive days. Mice were sacrificed by CO<sub>2</sub> inhalation at the end of the study and epididymal fat pads were excised for RNA extraction and analyzed as described above. Still further, serum protein levels were analyzed as described in the previous example

[0217] The results demonstrated that HAP and MCP-1 are reduced by rosiglitazone treatment at both the mRNA and protein levels (data not shown). Reduction of these markers corresponds with improvement in insulin response.

[0218] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.